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(54) **XANTHINE DEHYDROGENASE (XDH) IRNA COMPOSITIONS AND METHODS OF USE THEREOF**

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Related U.S. Application Data

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(60) Provisional application No. 62/287,522, filed on Jan. 27, 2016, provisional application No. 62/255,603, filed on Nov. 16, 2015, provisional application No. 62/197,221, filed on Jul. 27, 2015.

(51) **Int. Cl.**

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A61K 9/00	(2006.01)
A61K 31/713	(2006.01)
A61K 47/14	(2017.01)

(52) **U.S. Cl.**

CPC ***C12N 15/1137*** (2013.01); ***A61K 9/0019*** (2013.01); ***A61K 31/713*** (2013.01); ***A61K 47/14*** (2013.01); *C12N 2310/14* (2013.01); *C12N 2310/315* (2013.01); *C12N 2310/3125* (2013.01); *C12N 2310/321* (2013.01); *C12N 2310/322* (2013.01); *C12N 2310/335* (2013.01); *C12N 2310/351* (2013.01); *C12N 2310/3515* (2013.01)

(58) **Field of Classification Search**

CPC C12N 15/1137; C12N 2310/322; C12N 2310/335; C12N 2310/3515; C12N 2310/3125; C12N 2310/351; C12N 2310/14; C12N 2310/315; C12N 2310/321; A61K 9/0019; A61K 31/713; A61K 47/14

See application file for complete search history.

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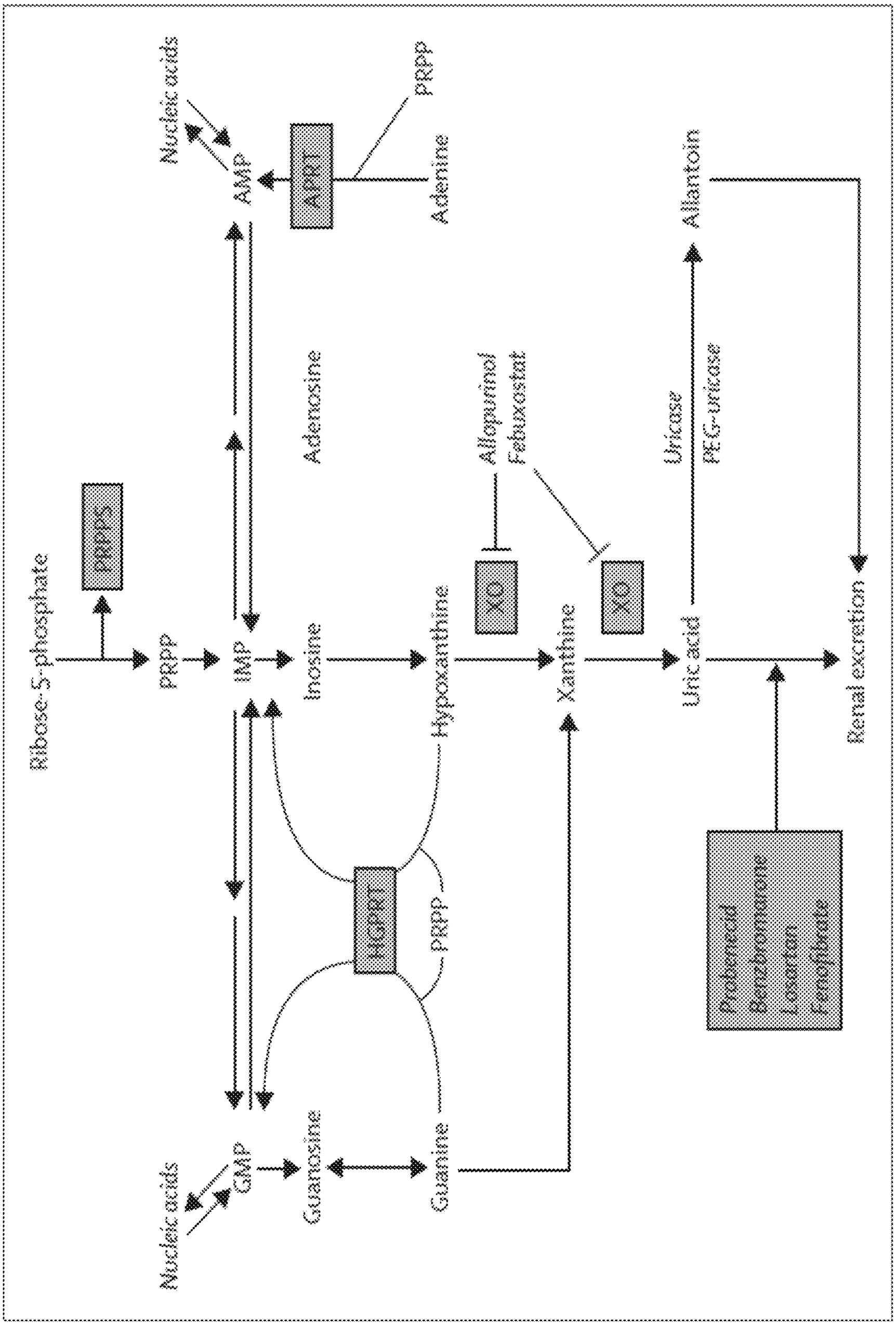
(57) **ABSTRACT**

The present invention relates to RNAi agents, e.g., double stranded RNAi agents, targeting a xanthine dehydrogenase (XDH) gene, and methods of using such double stranded RNAi agents to inhibit expression of an XDH gene and methods of treating subjects having an XDH-associated disease.

20 Claims, 1 Drawing Sheet

Specification includes a Sequence Listing.


Nucleic acids
AMP
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Ribose-5-phosphate
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Hypoxanthine
Allopurinol
Febuxostat
XO
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Xanthine
Uricase
Uric acid
PEG-uricase
Renal excretion
HGPRT
PRPP
Probenecid
Benzbromarone
Losartan
Fenofibrate
Nucleic acids
GMP
Guanosine
Guanine

XANTHINE DEHYDROGENASE (XDH) IRNA COMPOSITIONS AND METHODS OF USE THEREOF

RELATED APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 15/747,571, filed on Jan. 25, 2018, which is a 35 U.S.C. § 371 national stage filing of International Application No. PCT/US2016/043985, filed on Jul. 26, 2016, which in turn claims the benefit of priority to U.S. Provisional Application No. 62/287,522, filed on Jan. 27, 2016, U.S. Provisional Application No. 62/255,603, filed on Nov. 16, 2015, and U.S. Provisional Application No. 62/197,221, filed on Jul. 27, 2015. The entire contents of each of the foregoing applications are hereby incorporated herein by reference.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. The ASCII copy, created on Jan. 10, 2020, is named 121301_04005_SL.txt and is 600,559 bytes in size.

BACKGROUND OF THE INVENTION

Reduced renal clearance of uric acid is the result of a number of factors, including defects in uric acid transporter proteins such as SLC2A9, ABCG2, and others; reduced renal excretion due to renal disease, hypothyroidism, volume contraction and volume depletion, acidosis, lead intoxication, and familial nephropathy due to uromodulin deposits; and altered renal clearance due to hyperinsulinemia or insulin resistance in diabetes. Increased synthesis of uric acid is associated with hyperuricemia plus hyperuricosuria; inborn errors of metabolism such as Lesch Nyhan/HPRT deficiency, PRPP synthetase overactivity, and glucose-6-phosphate dehydrogenase deficiency (Von Gierke disease/Glycogen Storage Disease Type Ia); certain situations of high cell turnover (e.g., tumor lysis syndrome); certain situtations of high ATP turnover (e.g., glycogen storage diseases, tissue ischemia). Furthermore, conditions such as chronic kidney disease, hypertension, metabolic syndrome, and high fructose intake may result in both increased uric acid synthesis and decreased uric acid clearance.

Chronic elevated serum uric acid (chronic hyperuricemia), typically defined as serum urate levels greater than 6.8 mg/dl (greater than 360 mmol/), the level above which the physiological saturation threshold is exceeded (Mandell, *Cleve. Clin. Med.* 75: S5-S8, 2008), is associated with a number of diseases. For example, gout is characterized by recurrent attacks of acute inflammatory arthritis that is caused by an inflammatory reaction to uric acid crystals in the joint typically due to insufficient renal clearance of uric acid or excessive uric acid production. Fructose associated gout is associated with variants of transporters expressed in the kidney, intestine, and liver. Chronic elevated uric acid is also associated with non-alcoholic steatohepatitis (NASH), non-alcoholic fatty liver disease (NAFLD), metabolic disorder, cardiovascular disease, type 2 diabetes, and conditions linked to oxidative stress, chronic low grade inflammation, and insulin resistance (Xu et al., *J. Hepatol.* 62:1412-1419, 2015; Cardoso et al., *J. Pediatr.* 89:412-418, 2013; Sertoglu et al., *Clin. Biochem.,* 47:383-388, 2014).

Uric acid (also referred to herein as urate) is the final metabolite of endogenous and dietary purine metabolism. Xanthine oxidase (XO) (EC 1.1.3.22) and xanthine dehydrogenase (XDH) (EC 1.17.1.4), which catalyze the oxidation of hypoxanthine to xanthine, and xanthine to uric acid, respectively, are interconvertible forms of the same enzyme. The enzymes are molybdopterin-containing flavoproteins that consist of two identical subunits of approximately 145 kDa. The enzyme from mammalian sources, including man, is synthesized as the dehydrogenase form, but it can be readily converted to the oxidase form by oxidation of sulfhydryl residues or by proteolysis. XDH is primarily expressed in the intestine and the liver, but it is also expressed in other tissues including adipose tissue.

Allopurinol and febuxostat (Uloric®), inhibitors of the XDH form of the enzyme, are commonly used for the treatment of gout. However, their use is contraindicated in patients with co-morbidities common to gout, especially decreased renal function, e.g., due to chronic kidney disease or hepatic impairment. Their use may also be limited in patients with metabolic syndrome, hypertension, dyslipidemia, non-alcoholic steatohepatitis (NASH) or non-alcoholic fatty liver disease (NAFLD), cardiovascular disease, or diabetes (either type 1 or type 2), due to limited organ function from the disease or condition, or due to adverse drug interactions with agents used for the treatment of such conditions.

Currently, treatments for gout do not fully meet patient needs. Therefore, there is a need for additional therapies for subjects that would benefit from reduction in the expression of an XDH gene, such as a subject having an XDH-associated disease or disorder, e.g., gout.

SUMMARY OF THE INVENTION

The present invention provides iRNA compositions which affect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of an XDH gene. The XDH gene may be within a cell, e.g., a cell within a subject, such as a human.

In an aspect, the invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of a xanthine dehydrogenase (XDH) gene, wherein the dsRNA agent comprises a sense strand and an antisense strand, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:1 or 9 and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:2 or 10, respectively.

In certain embodiments, the sense strand and antisense strand independently comprise sequences selected from the group consisting of any one of the sequences in any one of Tables 3, 4, 6, and 7.

In an aspect, the invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of xanthine dehydrogenase (XDH), wherein the dsRNA agent comprises a sense strand and an antisense strand, the antisense strand comprising a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the antisense sequences listed in any one of Tables 3, 4, 6, and 7.

In certain embodiments, the dsRNA agent comprises at least one nucleotide comprising a nucleotide modification. In one embodiment, substantially all of the nucleotides of the sense strand comprise a modification. In another embodiment, substantially all of the nucleotides of the

antisense strand comprise a modification. In yet another embodiment, substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand comprise a modification. In one embodiment, all of the nucleotides of the sense strand comprise a modification. In another embodiment, all of the nucleotides of the antisense strand comprise a modification. In some embodiments, all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand comprise a nucleotide modification.

In an aspect, the invention provides a double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of a xanthine dehydrogenase (XDH) gene, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:1 or 9 and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:2 or 10, respectively, wherein substantially all of the nucleotides of the sense strand and substantially all of the nucleotides of the antisense strand comprise a nucleotide modification, and wherein the sense strand is conjugated to a ligand attached at the 3'-terminus.

In certain embodiments, the present invention provides double stranded ribonucleic acid (dsRNA) agents for inhibiting expression of a XDH gene, which comprise a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of nucleotides 490-512, 86-104, 273-291, 339-358, 410-428, 444-462, 490-509, 493-512, 888-907, 936-954, 969-987, 1105-1123, 1158-1176, 1242-1260, 1326-1344, 1357-1412, 1357-1379, 1357-1376, 1360-1379, 1378-1412, 1378-1396, 1394-1412, 1481-1499, 1515-1548, 1515-1533, 1530-1548, 1718-1736, 1783-1802, 1854-1890, 1854-1872, 1872-1890, 2053-2072, 2077-2096, 2137-2160, 2137-2156, 2142-2160, 2173-2206, 2173-2192, 2177-2195, 2184-2203, 2187-2206, 2314-2332, 2567-2604, 2567-2585, 2585-2604, 2620-2640, 2620-2639, 2621-2640, 2722-2740, 2891-2909, 2941-2975, 2941-2959, 2956-2975, 2993-3011, 3025-3061, 3025-3044, 3042-3061, 3062-3110, 3062-3080, 3079-3097, 3091-3110, 3112-3147, 3112-3130, 3129-3147, 3197-3215, 3247-3265, 3316-3334, 3366-3384, 3487-3520, 3487-3505, 3502-3520, 3606-3624, 3672-3690, 3891-3930, 3891-3910, 3893-3912, 3912-3930, 4063-4081, 4114-4132, 4152-4171, 4200-4218, 4300-4337, 4300-4319, 4303-4321, 4319-4337, 4386-4404, 4519-4538, 4541-4559, 4618-4637, or 4703-4722 of the nucleotide sequence of SEQ ID NO:1.

In certain embodiments, the present invention provides double stranded ribonucleic acid (dsRNA) agents for inhibiting expression of a XDH gene, which comprise a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides of any one of nucleotides 490-512, 86-104, 273-291, 339-358, 410-428, 444-462, 490-509, 493-512, 888-907, 936-954, 969-987, 1105-1123, 1158-1176, 1242-1260, 1326-1344, 1357-1412, 1357-1379, 1357-1376, 1360-1379, 1378-1412, 1378-1396, 1394-1412, 1481-1499, 1515-1548, 1515-1533, 1530-1548, 1718-1736, 1783-1802, 1854-1890, 1854-1872, 1872-1890, 2053-2072, 2077-2096, 2137-2160, 2137-2156, 2142-2160, 2173-2206, 2173-2192, 2177-2195, 2184-2203, 2187-2206, 2314-2332, 2567-2604, 2567-2585, 2585-2604, 2620-2640, 2620-2639, 2621-2640, 2722-2740, 2891-2909, 2941-2975, 2941-2959, 2956-2975, 2993-3011, 3025-3061, 3025-3044, 3042-3061, 3062-3110, 3062-3080, 3079-3097, 3091-3110, 3112-3147, 3112-3130, 3129-3147, 3197-3215, 3247-3265, 3316-3334, 3366-3384, 3487-3520, 3487-3505, 3502-3520, 3606-3624, 3672-3690, 3891-3930, 3891-3910, 3893-3912, 3912-3930, 4063-4081, 4114-4132, 4152-4171, 4200-4218, 4300-4337, 4300-4319, 4303-4321, 4319-4337, 4386-4404, 4519-4538, 4541-4559, 4618-4637, or 4703-4722 of the nucleotide sequence of SEQ ID NO: 1.

In certain embodiments, the sense strand and the antisense strand comprise a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the antisense sequences listed in any one of Tables 3, 4, 6, and 7. For example, in certain embodiments, the sense strand and the antisense strand comprise a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the antisense sequences of any one of the duplexes in any one of Tables 3, 4, 6 and 7. In certain embodiments, the sense strand and the antisense strand comprise a region of complementarity which comprises at least 15 contiguous nucleotides of any one of the antisense sequences of the duplexes any one of the duplexes in any one of Tables 3, 4, 6 and 7.

For example, in certain embodiments, the sense strand and the antisense strand comprise a region of complementarity which comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from any one of the antisense nucleotide sequences selected from the group consisting of the antisense nucleotide sequence of any of of duplexes AD-70016, AD-70033, AD-70053, AD-70050, AD-70051, AD-72007, AD-71990, AD-70049, AD-71993, AD-70042, AD-70052, AD-70018, AD-70055, AD-71831, AD-71810, AD-70023, AD-70035, AD-70044, AD-71820, AD-70027, AD-70034, AD-71837, AD-71861, AD-71998, AD-70020, AD-70026, AD-70030, AD-71801, AD-72003, AD-70025, AD-70021, AD-70029, AD-71834, AD-71765, AD-70043, AD-71840, AD-72006, AD-71844, AD-71779, AD-71830, AD-71952, AD-71766, AD-71950, AD-70022, AD-71833, AD-71823, AD-71847, AD-71900, AD-72014, AD-72015, AD-70037, AD-71982, AD-71787, AD-71894, AD-70048, AD-71887, AD-70028, AD-71980, AD-71826, AD-71855, AD-71778, AD-71757, AD-72012, AD-71854, AD-71890, AD-70038, AD-71865, AD-71933, AD-71942, AD-71901, AD-71878, AD-71905, and AD-71914.

In certain embodiments, the sense and antisense nucleotide sequences of a dsRNA agent of the invention are selected from the group consisting of a duplex selected from the group consisting of duplexes AD-70016, AD-70033, AD-70053, AD-70050, AD-70051, AD-72007, AD-71990, AD-70049, AD-71993, AD-70042, AD-70052, AD-70018, AD-70055, AD-71831, AD-71810, AD-70023, AD-70035, AD-70044, AD-71820, AD-70027, AD-70034, AD-71837, AD-71861, AD-71998, AD-70020, AD-70026, AD-70030, AD-71801, AD-72003, AD-70025, AD-70021, AD-70029, AD-71834, AD-71765, AD-70043, AD-71840, AD-72006, AD-71844, AD-71779, AD-71830, AD-71952, AD-71766, AD-71950, AD-70022, AD-71833, AD-71823, AD-71847, AD-71900, AD-72014, AD-72015, AD-70037, AD-71982, AD-71787, AD-71894, AD-70048, AD-71887, AD-70028, AD-71980, AD-71826, AD-71855, AD-71778, AD-71757, AD-72012, AD-71854, AD-71890, AD-70038, AD-71865, AD-71933, AD-71942, AD-71901, AD-71878, AD-71905, and AD-71914. In certain embodiments, the sense strand and the antisense strand comprise a region of complementarity which comprises at least 15 contiguous nucleotides of any one of the sense and antisense nucleotide sequences of any one of the foregoing duplexes.

In some embodiments, all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand comprise a nucleotide modification. In one embodiment, the

modified nucleotides are independently selected from the group consisting of a deoxy-nucleotide, a 3'-terminal deoxy-thymine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an unlocked nucleotide, a conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-O-allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, 2'-hydroxyl-modified nucleotide, a 2'-methoxyethyl modified nucleotide, a 2'-O-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified nucleotide, a nucleotide comprising a phosphorothioate group, a nucleotide comprising a methylphosphonate group, a nucleotide comprising a 5'-phosphate, and a nucleotide comprising a 5'-phosphate mimic. In another embodiment, the modified nucleotides comprise a short sequence of 3'-terminal deoxy-thymine nucleotides (dT), e.g., 1, 2, or 3 3'-terminal deoxy-thymine nucleotides (dT). In one embodiment, the modified nucleotides comprise two 3'-terminal deoxy-thymine nucleotides (dT) In certain embodiments, substantially all of the nucleotides of the sense strand are modified.

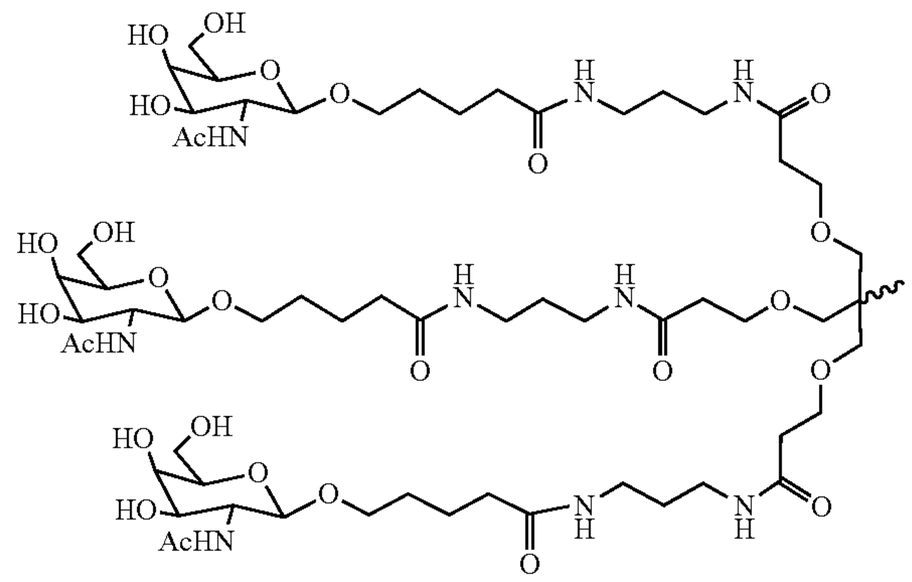
In certain embodiments, substantially all of the nucleotides of the antisense strand are modified. In certain embodiments, substantially all of the nucleotides of both the sense strand and the antisense strand are modified.

In certain embodiments, the duplex comprises, or consists of, a modified antisense strand nucleotide sequence provided in any one of Tables 4 and 7. In certain embodiments, the duplex comprises a modified sense strand nucleotide sequence provided in Table 4. In certain embodiments, the duplex comprises any one of the modified sense strand and antisense strand nucleotide sequences of a duplex provided in any one of Tables 4 and 7.

In certain embodiments, the region of complementarity between the antisense strand and the target is at least 17 nucleotides in length. For example, the region of complementarity between the antisense strand and the target is 19 to 21 nucleotides in length, for example, the region of complementarity is 21 nucleotides in length. In preferred embodiments, each strand is no more than 30 nucleotides in length.

In some embodiments, at least one strand comprises a 3' overhang of at least 1 nucleotide, e.g., at least one strand comprises a 3' overhang of at least 2 nucleotides.

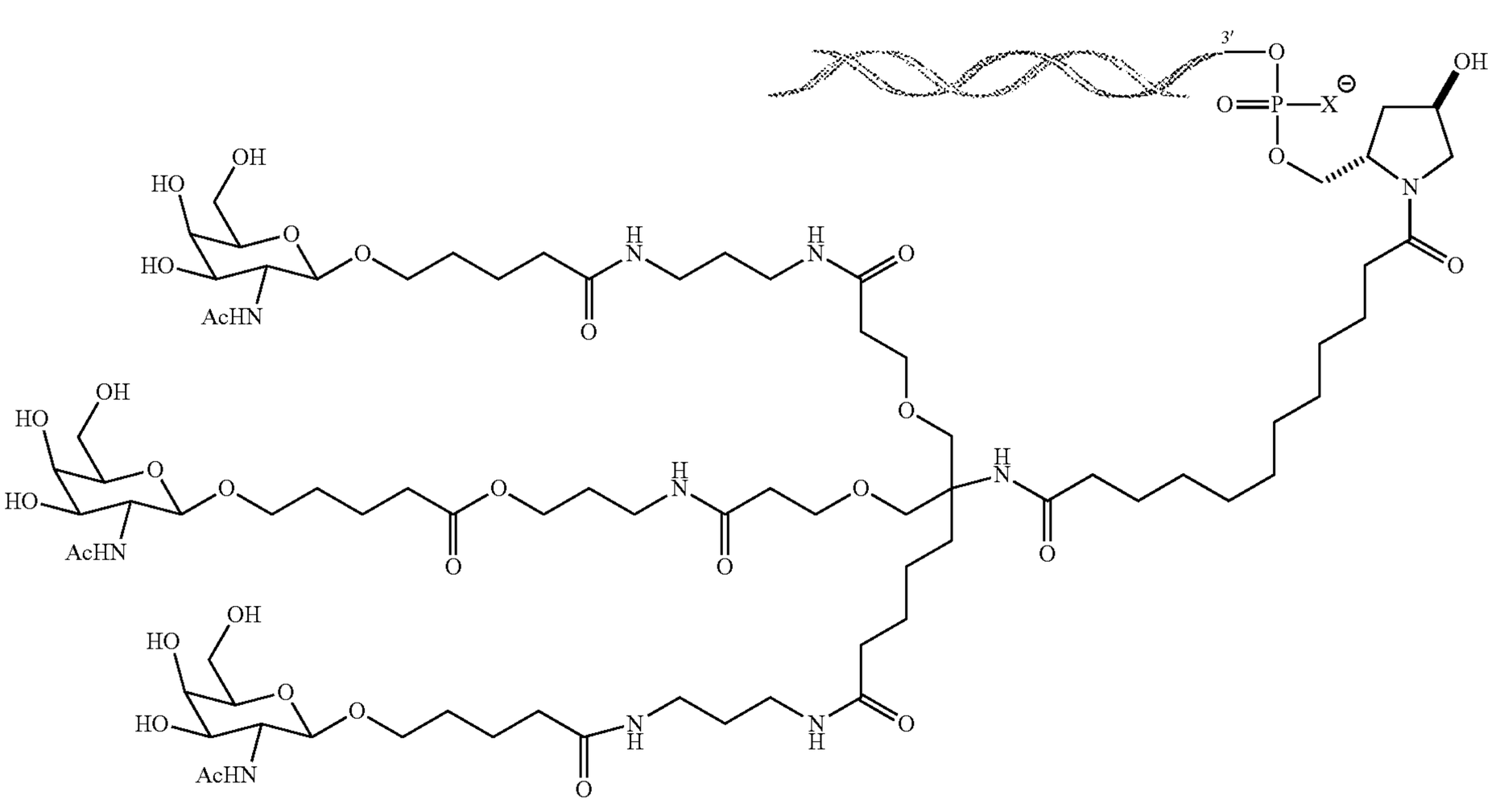
In many embodiments, the dsRNA agent further comprises a ligand. The ligand can be conjugated to the 3' end of the sense strand of the dsRNA agent. The ligand can be an N-acetylgalactosamine (GalNAc) derivative including, but not limited to



In various embodiments, the ligand is attached to the 5' end of the sense strand of the dsRNA agent, the 3' end of the antisense strand of the dsRNA agent, or the 5' end of the antisense strand of the dsRNA agent.

In some embodiments, the dsRNA agents of the invention comprise a plurality, e.g., 2, 3, 4, 5, or 6, of GalNAc, each independently attached to a plurality of nucleotides of the dsRNA agent through a plurality of monovalent linkers.

An exemplary dsRNA agent conjugated to the ligand as shown in the following schematic:



and, wherein X is O or S. In one embodiment, the X is O.

In certain embodiments, the ligand is a cholesterol.

In certain embodiments, the region of complementarity comprises any one of the antisense sequences in any one of Tables 3, 4, 6 and 7. In another embodiment, the region of complementarity consists of any one of the antisense sequences in any one of Tables 3, 4, 6 and 7.

In one embodiment, the dsRNA agent is selected from the group of dsRNA agents listed in any one of Tables 3, 4, 6, and 7.

In an aspect, the invention provides a double stranded ribonucleic acid (dsRNA) agents for inhibiting expression of a XDH gene, wherein the dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:1 or 9 and the antisense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of SEQ ID NO:2 or 10, respectively, wherein substantially all of the nucleotides of the sense strand comprise a nucleotide modification selected from a 2'-O-methyl modification and a 2'-fluoro modification, wherein the sense strand comprises two phosphorothioate internucleotide linkages at the 5'-terminus, wherein substantially all of the nucleotides of the antisense strand comprise a nucleotide modification selected from a 2'-O-methyl modification and a 2'-fluoro modification, wherein the antisense strand comprises two phosphorothioate internucleotide linkages at the 5'-terminus and two phosphorothioate internucleotide linkages at the 3'-terminus, and wherein the sense strand is conjugated to one or more GalNAc derivatives attached through a branched bivalent or trivalent linker at the 3'-terminus.

In certain embodiments, all of the nucleotides of the sense strand and all of the nucleotides of the antisense strand are modified nucleotides. In certain embodiments, each strand has 19-30 nucleotides.

In certain embodiments, substantially all of the nucleotides of the sense strand are modified. In certain embodiments, substantially all of the nucleotides of the antisense strand are modified. In certain embodiments, substantially all of the nucleotides of both the sense strand and the antisense strand are modified.

In an aspect, the invention provides a cell containing the dsRNA agent as described herein.

In an aspect, the invention provides a vector encoding at least one strand of a dsRNA agent, wherein the dsRNA agent comprises a region of complementarity to at least a part of an mRNA encoding XDH, wherein the dsRNA agent is 30 base pairs or less in length, and wherein the dsRNA agent targets the mRNA for cleavage. In certain embodiments, the region of complementarity is at least 15 nucleotides in length. In certain embodiments, the region of complementarity is 19 to 23 nucleotides in length.

In an aspect, the invention provides a cell comprising a vector as described herein.

In an aspect, the invention provides a pharmaceutical composition for inhibiting expression of an XDH gene comprising the dsRNA agent of the invention. In one embodiment, the dsRNA agent is administered in an unbuffered solution. In certain embodiments, the unbuffered solution is saline or water. In other embodiments, the dsRNA agent is administered with a buffer solution. In such embodiments, the buffer solution can comprises acetate, citrate, prolamine, carbonate, or phosphate, or any combination thereof. For example, the buffer solution can be phosphate buffered saline (PBS).

In an aspect, the invention provides a pharmaceutical composition comprising the dsRNA agent of the invention and a lipid formulation. In certain embodiments, the lipid formulation comprises a LNP. In certain embodiments, the lipid formulation comprises a MC3.

In an aspect, the invention provides a method of inhibiting XDH expression in a cell, the method comprising (a) contacting the cell with the dsRNA agent of the invention or the pharmaceutical composition of the invention; and (b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of an XDH gene, thereby inhibiting expression of the XDH gene in the cell. In certain embodiments, the cell is within a subject, for example, a human subject, for example a female human or a male human. In preferred embodiments, XDH expression is inhibited by at least 10%, 15%, 20%, preferably at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or to below the threshold of detection.

In an aspect, the invention provides a method of treating a subject having a disease or disorder that would benefit from reduction in XDH expression, the method comprising administering to the subject a therapeutically effective amount of the dsRNA agent of the invention or the pharmaceutical composition of the invention, thereby treating the subject.

In an aspect, the invention provides a method of preventing at least one symptom in a subject having a disease or disorder that would benefit from reduction in XDH expression, the method comprising administering to the subject a prophylactically effective amount of the dsRNA agent of the invention or a pharmaceutical composition of the invention, thereby preventing at least one symptom in the subject having a disorder that would benefit from reduction in XDH expression.

In certain embodiments, the administration of the dsRNA agent to the subject causes a decrease in the uric acid production. In certain embodiments, the administration of the dsRNA agent causes a decrease in the level of XDH in the subject, e.g., the level of XO or XDH protein, or XDH mRNA in the subject.

In certain embodiments, the XDH-associated disease is gout. In certain embodiments, the XDH-associated disease is Lesch Nyhan syndrome. In another embodiment, the XDH-associated disease is glycogen storage disease (GSD), e.g., GSD type Ia.

In certain embodiments, the invention further comprises administering an agent to decrease uric acid level in a subject, e.g., an inhibitor of uric acid production or an agent that increases uric acid elimination, to the subject with an XDH-associated disease. Agents to decrease the uric acid level in a subject include, but are not limited to, allopurinol, febuxostat, or an interleukin-1β (IL-1β) antagonist (canakinumab or rilonacept).

In certain embodiments, treatment of the subject with allopurinol is contraindicated. For example, administration of allopurinol is contraindicated in a subject with compromised renal function, due to, for example, chronic kidney disease, hypothyroidism, hyperinsulinemia, or insulin resistance. Administration of allopurinol may be contraindicated in combination with other drugs including, but not limited to, oral coagulants and probencid; subjects taking diuretics especially thiazide diuretics or other drugs that can reduce kidney function/have potential kidney toxicity.

In certain embodiments, the dsRNA agent is administered to a subject with an XDH-associated disease wherein the subject has compromised renal function. In certain embodiments, the dsRNA agent is administered to a subject with an XDH-associated disease wherein the subject is predisposed to compromised renal function, e.g., in a subject with hypertension, metabolic disorder, Type 1 diabetes, Type 2 diabetes, or the eldery. In certain embodiments, a subject is predisposed to compromised renal function as a result of treatment with one or more other therapeutic agents, e.g., diuretics.

In certain embodiments, the dsRNA agent is administered to a subject with an XDH-associated disease wherein the subject has failed treatment with allopurinol, e.g., gout flares during treatment, hypersensitivity reaction at any time after initiation of treatment with allopurinol or other unacceptable side effects as judged by the physician or patient.

Gout is often present in subjects who suffer from one or more co-morbidities. In certain embodiments, the dsRNA agent is administered to a subject with gout who has one or more of these co-morbidities. For example, in certain embodiments, the co-morbidity is reduced cardiac function or has cardiovascular disease. In certain embodiments, the co-morbidity is non-alcoholic fatty liver disease (NAFLD) or non-alcoholic steatohepatitis (NASH). In certain embodiments, the co-morbidity is metabolic syndrome. In certain embodiments, the co-morbidity is hyperlipidemia. In certain embodiments, the co-morbidity is reduced renal function (e.g., glomerular filtration rate of less than 60).

In certain embodiments if the invention, the dsRNA agent is administered to a subject with gout who is not obese.

In various embodiments, the dsRNA agent is administered at a dose of about 0.01 mg/kg to about 10 mg/kg or about 0.5 mg/kg to about 50 mg/kg. In some embodiments, the dsRNA agent is administered at a dose of about 10 mg/kg to about 30 mg/kg. In certain embodiments, the dsRNA agent is administered at a dose selected from 0.5 mg/kg, 1 mg/kg, 1.5 mg/kg, 3 mg/kg, 5 mg/kg, 10 mg/kg, and 30 mg/kg. In certain embodiments, the dsRNA agent is administered about once per week, once per month, once every other two months, or once a quarter (i.e., once every three months) at a dose of about 0.1 mg/kg to about 5.0 mg/kg.

In certain embodiments, the dsRNA agent is administered to the subject once a week. In certain embodiments, the dsdsRNA agent is administered to the subject once a month. In certain embodiments, the dsRNA agent is administered once per quarter (i.e., every three months).

In some embodiment, the dsdsRNA agent is administered to the subject subcutaneously.

In various embodiments, the methods of the invention further comprise monitoring the subject for at least one sign or symptom of an XDH-related disease, for example, gout.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** is a schematic showing the uric acid metabolic pathway. XDH is labeled as XO in the schematic.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of a xanthine dehydrogenase (XDH) gene. The gene may be within a cell, e.g., a cell within a subject, such as a human. The use of these iRNAs enables the targeted degradation of mRNAs of the corresponding gene (XDH gene) in mammals.

The iRNAs of the invention have been designed to target the human XDH gene, including portions of the gene that are conserved in the XDH othologs of other mammalian species. Without intending to be limited by theory, it is believed that the specific target sites or the specific modifications in these iRNAs confer to the iRNAs of the invention improved efficacy, stability, potency, durability, and safety.

Accordingly, the present invention also provides methods for treating a subject having a disorder that would benefit from inhibiting or reducing the expression of an XDH gene, e.g., an XDH-associated disease, such as gout, using iRNA compositions which effect the RNA-induced silencing complex (RISC)-mediated cleavage of RNA transcripts of an XDH gene.

The iRNAs of the invention include an RNA strand (the antisense strand) having a region which is about 30 nucleotides or less in length, e.g., 15-30, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length, which region is substantially complementary to at least part of an mRNA transcript of an XDH gene. In certain embodiments, the iRNAs of the invention include an RNA strand (the antisense strand) which can include longer lengths, for example up to 66 nucleotides, e.g., 36-66, 26-36, 25-36, 31-60, 22-43, 27-53 nucleotides in length with a region of at least 19 contiguous nucleotides that is substantially complementary to at least a part of an mRNA transcript of an XDH gene. These iRNAs with the longer length antisense strands preferably include a second RNA strand (the sense strand) of 20-60 nucleotides in length wherein the sense and antisense strands form a duplex of 18-30 contiguous nucleotides.

The use of the iRNA agents described herein enable the targeted degradation of mRNAs of the corresponding gene (XDH gene) in mammals. Very low dosages of the iRNAs of the invention, in particular, can specifically and efficiently mediate RNA interference (RNAi), resulting in significant inhibition of expression of the corresponding gene (XDH gene). Using in vitro and in vivo assays, the present inventors have demonstrated that iRNAs targeting an XDH gene can mediate RNAi, resulting in significant inhibition of expression of XDH and a decrease in uric acid levels. Thus, methods and compositions including these iRNAs are useful for treating a subject having an XDH-associated disease, such as gout.

The following detailed description discloses how to make and use compositions containing iRNAs to inhibit the expression of an XDH gene as well as compositions, uses, and methods for treating subjects having diseases and disorders that would benefit from reduction of the expression of an XDH gene.

I. Definitions

In order that the present invention may be more readily understood, certain terms are first defined. In addition, it should be noted that whenever a value or range of values of a parameter are recited, it is intended that values and ranges intermediate to the recited values are also intended to be part of this invention.

The articles "a" and "an" are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element, e.g., a plurality of elements.

The term "including" is used herein to mean, and is used interchangeably with, the phrase "including but not limited to".

The term "or" is used herein to mean, and is used interchangeably with, the term "and/or," unless context clearly indicates otherwise. For example, "sense strand or antisense strand" is understood as "sense strand or antisense strand or sense strand and antisense strand."

The term "about" is used herein to mean within the typical ranges of tolerances in the art. For example, "about" can be understood as about 2 standard deviations from the mean. In certain embodiments, about means±10%. In certain embodiments, about means±5%. When about is present before a series of numbers or a range, it is understood that "about" can modify each of the numbers in the series or range.

The term "at least" prior to a number or series of numbers is understood to include the number adjacent to the term "at least", and all subsequent numbers or integers that could logically be included, as clear from context. For example, the number of nucleotides in a nucleic acid molecule must be an integer. For example, "at least 18 nucleotides of a 21 nucleotide nucleic acid molecule" means that 18, 19, 20, or 21 nucleotides have the indicated property. When at least is present before a series of numbers or a range, it is understood that "at least" can modify each of the numbers in the series or range.

As used herein, "no more than" or "less than" is understood as the value adjacent to the phrase and logical lower values or intergers, as logical from context, to zero. For example, a duplex with an overhang of "no more than 2 nucleotides" has a 2, 1, or 0 nucleotide overhang. When "no more than" is present before a series of numbers or a range, it is understood that "no more than" can modify each of the numbers in the series or range.

As used herein, ranges include both the upper and lower limit.

In the event of a conflict between a sequence and its indicated site on a transcript or other sequence, the nucleotide sequence recited in the specification takes precedence.

Various embodiments of the invention can be combined as determined appropriate by one of skill in the art.

"Xanthine dehydrogenase" or "XDH" belongs to the group of molybdenum-containing hydroxylases involved in the oxidative metabolism of purines. The encoded protein has been identified as a moonlighting protein based on its ability to perform mechanistically distinct functions. Xanthine dehydrogenase can be converted to xanthine oxidase by reversible sulfhydryl oxidation or by irreversible proteolytic modification. As used herein, unless clear from context, xanthine dehydrogenase or XDH is understood to include both the xanthine dehydrogenase and xanthine oxidase ("XO" or "XOR") form of the protein. The protein is expressed predominantly in the intestine and the liver, but is also expressed in adipose tissue. Two transcript variants have been identified for the human isoform of the gene. Further information on XDH is provided, for example in the NCBI Gene database at www.ncbi.nlm.nih.gov/gene/7498 (which is incorporated herein by reference as of the date of filing this application). The amino acid and complete coding sequences of the reference sequence of the human XDH gene may be found in, for example, GenBank Accession No. GI: 91823270 (RefSeq Accession No. NM_000379.3; SEQ ID NO:1; the reverse complement of SEQ ID NO:1 is shown in SEQ ID NO:2) and GenBank Accession No. GI: 767915203 (RefSeq Accession No. XM_011533096; SEQ ID NO: 9; the reverse complement of SEQ ID NO:9 is shown in SEQ ID NO:10). The nucleotide and amino acid sequence of mammalian orthologs of the human XDH gene may be found in, for example, GI: 575501724 (RefSeq Accession No. NM_011723.3, mouse; SEQ ID NO:3; the reverse complement of SEQ ID NO:3 is shown in SEQ ID NO:4); GI: 8394543 (RefSeq Accession No. NM_017154.1, rat; SEQ ID NO:5; the reverse complement of SEQ ID NO:5 is shown in SEQ ID NO:6); GenBank Accession Nos. GI: 544482046 (RefSeq Accession Nos. XM_005576183.1 and XM_005576184.1, cynomolgus monkey; SEQ ID NO:7; the reverse complement of SEQ ID NO:7 is shown in SEQ ID NO:8, and SEQ ID NO:15; the reverse complement of SEQ ID NO:15 is shown in SEQ ID NO:16, respectively).

A number of naturally occurring SNPs in the XDH gene are known and can be found, for example, in the SNP database at the NCBI at http://www.ncbi.nlm.nih.gov/snp?LinkName=gene_snp&from_uid=7498 (which is incorporated herein by reference as of the date of filing this application) which lists over 3000 SNPs in human XDH. In preferred embodiments, such naturally occurring variants are included within the scope of the XDH gene sequence.

Additional examples of XDH mRNA sequences are readily available using publicly available databases, e.g., GenBank, UniProt, and OMIM.

As used herein, "target sequence" refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of an XDH gene, including mRNA that is a product of RNA processing of a primary transcription product. The target portion of the sequence will be at least long enough to serve as a substrate for iRNA-directed cleavage at or near that portion of the nucleotide sequence of an mRNA molecule formed during the transcription of an XDH gene. In one embodiment, the target sequence is within the protein coding region of XDH.

The target sequence may be from about 9-36 nucleotides in length, e.g., about 15-30 nucleotides in length. For example, the target sequence can be from about 15-30 nucleotides, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

As used herein, the term "strand comprising a sequence" refers to an oligonucleotide comprising a chain of nucleotides that is described by the sequence referred to using the standard nucleotide nomenclature.

"G," "C," "A," "T," and "U" each generally stand for a nucleotide that contains guanine, cytosine, adenine, thymidine, and uracil as a base, respectively. However, it will be understood that the term "ribonucleotide" or "nucleotide" can also refer to a modified nucleotide, as further detailed below, or a surrogate replacement moiety (see, e.g., Table 2). The skilled person is well aware that guanine, cytosine, adenine, and uracil can be replaced by other moieties without substantially altering the base pairing properties of an oligonucleotide comprising a nucleotide bearing such replacement moiety. For example, without limitation, a nucleotide comprising inosine as its base can base pair with

nucleotides containing adenine, cytosine, or uracil. Hence, nucleotides containing uracil, guanine, or adenine can be replaced in the nucleotide sequences of dsRNA featured in the invention by a nucleotide containing, for example, inosine. In another example, adenine and cytosine anywhere in the oligonucleotide can be replaced with guanine and uracil, respectively to form G-U Wobble base pairing with the target mRNA. Sequences containing such replacement moieties are suitable for the compositions and methods featured in the invention.

The terms "iRNA," "RNAi agent," "iRNA agent," "RNA interference agent" as used interchangeably herein, refer to an agent that contains RNA as that term is defined herein, and which mediates the targeted cleavage of an RNA transcript via an RNA-induced silencing complex (RISC) pathway. iRNA directs the sequence-specific degradation of mRNA through a process known as RNA interference (RNAi). The iRNA modulates, e.g., inhibits, the expression of an XDH gene in a cell, e.g., a cell within a subject, such as a mammalian subject.

In one embodiment, an RNAi agent of the invention includes a single stranded RNAi that interacts with a target RNA sequence, e.g., an XDH target mRNA sequence, to direct the cleavage of the target RNA. Without wishing to be bound by theory it is believed that long double stranded RNA introduced into cells is broken down into double stranded short interfering RNAs (siRNAs) comprising a sense strand and an antisense strand by a Type III endonuclease known as Dicer (Sharp et al. (2001) *Genes Dev.* 15:485). Dicer, a ribonuclease-III-like enzyme, processes these dsRNA into 19-23 base pair short interfering RNAs with characteristic two base 3' overhangs (Bernstein, et al., (2001) *Nature* 409:363). These siRNAs are then incorporated into an RNA-induced silencing complex (RISC) where one or more helicases unwind the siRNA duplex, enabling the complementary antisense strand to guide target recognition (Nykanen, et al., (2001) *Cell* 107:309). Upon binding to the appropriate target mRNA, one or more endonucleases within the RISC cleave the target to induce silencing (Elbashir, et al., (2001) *Genes Dev.* 15:188). Thus, in one aspect the invention relates to a single stranded RNA (ssRNA) (the antisense strand of an siRNA duplex) generated within a cell and which promotes the formation of a RISC complex to effect silencing of the target gene, i.e., an XDH gene. Accordingly, the term "siRNA" is also used herein to refer to an RNAi as described above.

In another embodiment, the RNAi agent may be a single-stranded RNA that is introduced into a cell or organism to inhibit a target mRNA. Single-stranded RNAi agents bind to the RISC endonuclease, Argonaute 2, which then cleaves the target mRNA. The single-stranded siRNAs are generally 15-30 nucleotides in length and are chemically modified. The design and testing of single-stranded RNAs are described in U.S. Pat. No. 8,101,348 and in Lima et al., (2012) *Cell* 150:883-894, the entire contents of each of which are hereby incorporated herein by reference. Any of the antisense nucleotide sequences described herein may be used as a single-stranded siRNA as described herein or as chemically modified by the methods described in Lima et al., (2012) *Cell* 150:883-894.

In certain embodiments, an "iRNA" for use in the compositions, uses, and methods of the invention is a double stranded RNA and is referred to herein as a "double stranded RNAi agent," "double stranded RNA (dsRNA) molecule," "dsRNA agent," or "dsRNA". The term "dsRNA" refers to a complex of ribonucleic acid molecules, having a duplex structure comprising two anti-parallel and substantially complementary nucleic acid strands, referred to as having "sense" and "antisense" orientations with respect to a target RNA, i.e., an XDH gene. In some embodiments of the invention, a double stranded RNA (dsRNA) triggers the degradation of a target RNA, e.g., an mRNA, through a post-transcriptional gene-silencing mechanism referred to herein as RNA interference or RNAi.

In general, the majority of nucleotides of each strand of a dsRNA molecule are ribonucleotides, but as described in detail herein, each or both strands can also include one or more non-ribonucleotides, e.g., a deoxyribonucleotide or a modified nucleotide. In addition, as used in this specification, an "iRNA" may include ribonucleotides with chemical modifications; an iRNA may include substantial modifications at multiple nucleotides.

As used herein, the term "modified nucleotide" refers to a nucleotide having, independently, a modified sugar moiety, a modified internucleotide linkage, or modified nucleobase, or any combination thereof. Thus, the term modified nucleotide encompasses substitutions, additions or removal of, e.g., a functional group or atom, to internucleoside linkages, sugar moieties, or nucleobases. The modifications suitable for use in the agents of the invention include all types of modifications disclosed herein or known in the art. Any such modifications, as used in a siRNA type molecule, are encompassed by "iRNA" or "RNAi agent" for the purposes of this specification and claims.

The duplex region may be of any length that permits specific degradation of a desired target RNA through a RISC pathway, and may range from about 9 to 36 base pairs in length, e.g., about 15-30 base pairs in length, for example, about 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36 base pairs in length, such as about 15-30, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

The two strands forming the duplex structure may be different portions of one larger RNA molecule, or they may be separate RNA molecules. Where the two strands are part of one larger molecule, and therefore are connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming the duplex structure, the connecting RNA chain is referred to as a "hairpin loop." A hairpin loop can comprise at least one unpaired nucleotide. In some embodiments, the hairpin loop can comprise at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 23 or more unpaired nucleotides. In some embodiments, the hairpin loop can be 10 or fewer nucleotides. In some embodiments, the hairpin loop can be 8 or fewer unpaired nucleotides. In some embodiments, the hairpin loop can be 4-10 unpaired nucleotides. In some embodiments, the hairpin loop can be 4-8 nucleotides.

Where the two substantially complementary strands of a dsRNA are comprised by separate RNA molecules, those molecules need not, but can be covalently connected. Where the two strands are connected covalently by means other than an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming the duplex structure, the connecting structure is referred to as a "linker." The RNA strands may have the

same or a different number of nucleotides. The maximum number of base pairs is the number of nucleotides in the shortest strand of the dsRNA minus any overhangs that are present in the duplex. In addition to the duplex structure, an RNAi may comprise one or more nucleotide overhangs.

In certain embodiments, an iRNA agent of the invention is a dsRNA, each strand of which comprises 19-23 nucleotides, that interacts with a target RNA sequence, e.g., an XDH gene. Without wishing to be bound by theory, long double stranded RNA introduced into cells is broken down into siRNA by a Type III endonuclease known as Dicer (Sharp et al. (2001) Genes Dev. 15:485). Dicer, a ribonuclease-III-like enzyme, processes the dsRNA into 19-23 base pair short interfering RNAs with characteristic two base 3' overhangs (Bernstein, et al., (2001) Nature 409:363). The siRNAs are then incorporated into an RNA-induced silencing complex (RISC) where one or more helicases unwind the siRNA duplex, enabling the complementary antisense strand to guide target recognition (Nykanen, et al., (2001) Cell 107:309). Upon binding to the appropriate target mRNA, one or more endonucleases within the RISC cleave the target to induce silencing (Elbashir, et al., (2001) Genes Dev. 15:188).

In some embodiments, an iRNA of the invention is a dsRNA of 24-30 nucleotides that interacts with a target RNA sequence, e.g., an XDH target mRNA sequence, to direct the cleavage of the target RNA. Without wishing to be bound by theory, long double stranded RNA introduced into cells is broken down into siRNA by a Type III endonuclease known as Dicer (Sharp et al. (2001) *Genes Dev.* 15:485). Dicer, a ribonuclease-III-like enzyme, processes the dsRNA into 19-23 base pair short interfering RNAs with characteristic two base 3' overhangs (Bernstein, et al., (2001) *Nature* 409:363). The siRNAs are then incorporated into an RNA-induced silencing complex (RISC) where one or more helicases unwind the siRNA duplex, enabling the complementary antisense strand to guide target recognition (Nykanen, et al., (2001) *Cell* 107:309). Upon binding to the appropriate target mRNA, one or more endonucleases within the RISC cleave the target to induce silencing (Elbashir, et al., (2001) *Genes Dev.* 15:188).

As used herein, the term "nucleotide overhang" refers to at least one unpaired nucleotide that protrudes from the duplex structure of a double strainded iRNA. For example, when a 3'-end of one strand of a dsRNA extends beyond the 5'-end of the other strand, or vice versa, there is a nucleotide overhang. A dsRNA can comprise an overhang of at least one nucleotide; alternatively the overhang can comprise at least two nucleotides, e.g., 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, or 15 nucleotides. In certain embodiments, the overhang can be at least three nucleotides, at least four nucleotides, at least five nucleotides or more. A nucleotide overhang can comprise or consist of a nucleotide/nucleoside analog, including a deoxynucleotide/nucleoside. The overhang(s) can be on the sense strand, the antisense strand, or any combination thereof. Furthermore, the nucleotide(s) of an overhang can be present on the 5'-end, 3'-end, or both ends of either an antisense or sense strand of a dsRNA.

In certain embodiments, the antisense strand of a dsRNA has a 1-10 nucleotide, e.g., a 1-3, 1-4, 2-4, 1-5, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 nucleotide, overhang at the 3'-end or the 5'-end. In certain embodiments, the overhang on the sense strand or the antisense strand, or both, can include extended lengths longer than 10 nucleotides, e.g., 1-30 nucleotides, 2-30 nucleotides, 10-30 nucleotides, or 10-15 nucleotides in length. In certain embodiments, an extended overhang is on the sense strand of the duplex. In certain embodiments, an extended overhang is present on the 3' end of the sense strand of the duplex. In certain embodiments, an extended overhang is present on the 5' end of the sense strand of the duplex. In certain embodiments, an extended overhang is on the antisense strand of the duplex. In certain embodiments, an extended overhang is present on the 3' end of the antisense strand of the duplex. In certain embodiments, an extended overhang is present on the 5' end of the antisense strand of the duplex. In certain embodiments, one or more of the nucleotides in the overhang is replaced with a nucleoside thiophosphate.

"Blunt" or "blunt end" means that there are no unpaired nucleotides at that end of the double stranded RNAi agent, i.e., no nucleotide overhang. A "blunt ended" double stranded RNAi agent is double stranded over its entire length, i.e., no nucleotide overhang at either end of the molecule. The RNAi agents of the invention include RNAi agents with no nucleotide overhang at one end (i.e., agents with one overhang and one blunt end) or with no nucleotide overhangs at either end.

The term "antisense strand" or "guide strand" refers to the strand of an iRNA, e.g., a dsRNA, which includes a region that is substantially complementary to a target sequence, e.g., an XDH mRNA. As used herein, the term "region of complementarity" refers to the region on the antisense strand that is substantially complementary to a sequence, for example a target sequence, e.g., an XDH nucleotide sequence, as defined herein. Where the region of complementarity is not fully complementary to the target sequence, the mismatches can be in the internal or terminal regions of the molecule. Generally, the most tolerated mismatches are in the terminal regions, e.g., within 5, 4, 3, 2, or 1 nucleotides of the 5'- or 3'-end of the iRNA. In some embodiments, a double stranded RNAi agent of the invention includes a nucleotide mismatch in the antisense strand. In some embodiments, a double stranded RNAi agent of the invention includes a nucleotide mismatch in the sense strand. In some embodiments, the nucleotide mismatch is, for example, within 5, 4, 3, 2, or 1 nucleotides from the 3'-end of the iRNA. In another embodiment, the nucleotide mismatch is, for example, in the 3'-terminal nucleotide of the iRNA.

The term "sense strand" or "passenger strand" as used herein, refers to the strand of an iRNA that includes a region that is substantially complementary to a region of the antisense strand as that term is defined herein.

As used herein, "substantially all of the nucleotides are modified" are largely but not wholly modified and can include not more than 5, 4, 3, 2, or 1 unmodified nucleotides.

As used herein, the term "cleavage region" refers to a region that is located immediately adjacent to the cleavage site. The cleavage site is the site on the target at which cleavage occurs. In some embodiments, the cleavage region comprises three bases on either end of, and immediately adjacent to, the cleavage site. In some embodiments, the cleavage region comprises two bases on either end of, and immediately adjacent to, the cleavage site. In some embodiments, the cleavage site specifically occurs at the site bound by nucleotides 10 and 11 of the antisense strand, and the cleavage region comprises nucleotides 11, 12 and 13.

As used herein, and unless otherwise indicated, the term "complementary," when used to describe a first nucleotide sequence in relation to a second nucleotide sequence, refers to the ability of an oligonucleotide or polynucleotide comprising the first nucleotide sequence to hybridize and form a duplex structure under certain conditions with an oligonucleotide or polynucleotide comprising the second nucleo-

tide sequence, as will be understood by the skilled person. Such conditions can, for example, be stringent conditions, where stringent conditions can include: 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 50° C. or 70° C. for 12-16 hours followed by washing (see, e.g., "Molecular Cloning: A Laboratory Manual, Sambrook, et al. (1989) Cold Spring Harbor Laboratory Press). Other conditions, such as physiologically relevant conditions as can be encountered inside an organism, can apply. The skilled person will be able to determine the set of conditions most appropriate for a test of complementarity of two sequences in accordance with the ultimate application of the hybridized nucleotides.

Complementary sequences within an iRNA, e.g., within a dsRNA as described herein, include base-pairing of the oligonucleotide or polynucleotide comprising a first nucleotide sequence to an oligonucleotide or polynucleotide comprising a second nucleotide sequence over the entire length of one or both nucleotide sequences. Such sequences can be referred to as "fully complementary" with respect to each other herein. However, where a first sequence is referred to as "substantially complementary" with respect to a second sequence herein, the two sequences can be fully complementary, or they can form one or more, but generally not more than 5, 4, 3, or 2 mismatched base pairs upon hybridization for a duplex up to 30 base pairs, while retaining the ability to hybridize under the conditions most relevant to their ultimate application, e.g., inhibition of gene expression via a RISC pathway. However, where two oligonucleotides are designed to form, upon hybridization, one or more single stranded overhangs, such overhangs shall not be regarded as mismatches with regard to the determination of complementarity. For example, a dsRNA comprising one oligonucleotide 21 nucleotides in length and another oligonucleotide 23 nucleotides in length, wherein the longer oligonucleotide comprises a sequence of 21 nucleotides that is fully complementary to the shorter oligonucleotide, can yet be referred to as "fully complementary" for the purposes described herein.

"Complementary" sequences, as used herein, can also include, or be formed entirely from, non-Watson-Crick base pairs or base pairs formed from non-natural and modified nucleotides, in so far as the above requirements with respect to their ability to hybridize are fulfilled. Such non-Watson-Crick base pairs include, but are not limited to, G:U Wobble or Hoogstein base pairing.

The terms "complementary," "fully complementary," and "substantially complementary" herein can be used with respect to the base matching between the sense strand and the antisense strand of a dsRNA, or between the antisense strand of a double stranded RNAi agent and a target sequence, as will be understood from the context of their use.

As used herein, a polynucleotide that is "substantially complementary to at least part of" a messenger RNA (mRNA) refers to a polynucleotide that is substantially complementary to a contiguous portion of the mRNA of interest (e.g., an mRNA encoding an XDH gene). For example, a polynucleotide is complementary to at least a part of an XDH mRNA if the sequence is substantially complementary to a non-interrupted portion of an mRNA encoding an XDH gene.

Accordingly, in some embodiments, the antisense polynucleotides disclosed herein are fully complementary to the target XDH sequence. In some embodiments, the sense polynucleotides disclosed herein are fully complementary to the antisense sequence of a target XDH sequence.

In other embodiments, the antisense polynucleotides disclosed herein are substantially complementary to the target XDH sequence and comprise a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to the equivalent region of the nucleotide sequence of SEQ ID NO:1 or 2, or a fragment of SEQ ID NO:1 or 2, such as about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about % 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary.

In other embodiments, the sense polynucleotides disclosed herein are substantially complementary to the antisense sequence of a target XDH sequence and comprise a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to the equivalent region of the nucleotide sequence of SEQ ID NO:2, or a fragment of SEQ ID NO:2, such as about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about % 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary.

In one embodiment, an RNAi agent of the invention includes a sense strand that is substantially complementary to an antisense polynucleotide which, in turn, is complementary to a target XDH sequence and comprises a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to any one of the sense strand nucleotide sequences in any one of Tables 3, 4, 6 and 7, or a fragment of any one of the sense strand nucleotide sequences in any one of Tables 3, 4, 6 and 7, such as about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about % 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% complementary.

In some embodiments, an iRNA of the invention includes an antisense strand that is substantially complementary to the target XDH sequence and comprises a contiguous nucleotide sequence which is at least about 80% complementary over its entire length to the equivalent region of the nucleotide sequence of any one of the antisense strand nucleotide sequences in any one of Tables 3, 4, 6 and 7, or a fragment of any one of the antisense strand nucleotide sequences in any one of Tables 3, 4, 6 and 7, such as at least 85%, 90%, 95% complementary, or 100% complementary.

In an aspect of the invention, an agent for use in the methods and compositions of the invention is a single-stranded antisense oligonucleotide molecule that inhibits a target mRNA via an antisense inhibition mechanism. The single-stranded antisense oligonucleotide molecule is complementary to a sequence within the target mRNA. The single-stranded antisense oligonucleotides can inhibit translation in a stoichiometric manner by base pairing to the mRNA and physically obstructing the translation machinery, see Dias, N. et al., (2002) *Mol Cancer Ther* 1:347-355. The single-stranded antisense oligonucleotide molecule may be about 14 to about 30 nucleotides in length and have a sequence that is complementary to a target sequence. For example, the single-stranded antisense oligonucleotide molecule may comprise a sequence that is at least about 14, 15, 16, 17, 18, 19, 20, or more contiguous nucleotides from any one of the antisense sequences described herein.

The phrase "contacting a cell with an iRNA," such as a dsRNA, as used herein, includes contacting a cell by any possible means. Contacting a cell with an iRNA includes contacting a cell in vitro with the iRNA or contacting a cell in vivo with the iRNA. The contacting may be done directly or indirectly. Thus, for example, the iRNA may be put into physical contact with the cell by the individual performing

the method, or alternatively, the iRNA may be put into a situation that will permit or cause it to subsequently come into contact with the cell.

Contacting a cell in vitro may be done, for example, by incubating the cell with the iRNA. Contacting a cell in vivo may be done, for example, by injecting the iRNA into or near the tissue where the cell is located, or by injecting the iRNA into another area, e.g., the bloodstream or the subcutaneous space, such that the agent will subsequently reach the tissue where the cell to be contacted is located. For example, the iRNA may contain or be coupled to a ligand, e.g., GalNAc3, that directs the iRNA to a site of interest, e.g., the liver. Combinations of in vitro and in vivo methods of contacting are also possible. For example, a cell may also be contacted in vitro with an iRNA and subsequently transplanted into a subject.

In certain embodiments, contacting a cell with an iRNA includes "introducing" or "delivering the iRNA into the cell" by facilitating or effecting uptake or absorption into the cell. Absorption or uptake of an iRNA can occur through unaided diffusion or active cellular processes, or by auxiliary agents or devices. Introducing an iRNA into a cell may be in vitro or in vivo. For example, for in vivo introduction, iRNA can be injected into a tissue site or administered systemically. In vivo delivery can also be done by a beta-glucan delivery system, such as those described in U.S. Pat. Nos. 5,032,401 and 5,607,677, and US Publication No. 2005/0281781, the entire contents of which are hereby incorporated herein by reference. In vitro introduction into a cell includes methods known in the art such as electroporation and lipofection. Further approaches are described herein below or are known in the art.

The term "lipid nanoparticle" or "LNP" is a vesicle comprising a lipid layer encapsulating a pharmaceutically active molecule, such as a nucleic acid molecule, e.g., an iRNA or a plasmid from which an iRNA is transcribed. LNPs are described in, for example, U.S. Pat. Nos. 6,858,225, 6,815,432, 8,158,601, and 8,058,069, the entire contents of which are hereby incorporated herein by reference.

As used herein, a "subject" is an animal, such as a mammal, including a primate (such as a human, a non-human primate, e.g., a monkey, and a chimpanzee), a non-primate (such as a cow, a pig, a camel, a llama, a horse, a goat, a rabbit, a sheep, a hamster, a guinea pig, a cat, a dog, a rat, a mouse, and a horse), or a bird (e.g., a duck or a goose) that expresses the target gene, either endogenously or heterologously. In certain embodiments, the subject is a human, such as a human being treated or assessed for a disease, disorder or condition that would benefit from reduction in XDH gene expression or replication; a human at risk for a disease, disorder or condition that would benefit from reduction in XDH gene expression; a human having a disease, disorder or condition that would benefit from reduction in XDH gene expression; or human being treated for a disease, disorder or condition that would benefit from reduction in XDH gene expression, as described herein. In some embodiments, the subject is a female human. In other embodiments, the subject is a male human.

As used herein, the terms "treating" or "treatment" refer to a beneficial or desired result including, but not limited to, alleviation or amelioration of one or more symptoms associated with XDH gene expression or XDH protein production, e.g., gout, NASH, or NAFLD. "Treatment" can also mean prolonging survival as compared to expected survival in the absence of treatment.

The term "lower" in the context of the level of XDH gene expression or XDH protein production in a subject, or a disease marker or symptom refers to a statistically significant decrease in such level. The decrease can be, for example, at least 20%, 25%, preferably at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%, or below the level of detection for the detection method. In certain embodiments, the expression of the target is normalized, i.e., decreased to a level accepted as within the range of normal for an individual without such disorder. For example, chronic hyperuricemia is defined as serum urate levels greater than 6.8 mg/dl (greater than 360 mmol/), the level above which the physiological saturation threshold is exceeded (Mandell, Cleve. Clin. Med. 75:S5-S8, 2008). In certain embodiments, the reduction is the normalization of the level of a sign or symptom of a disease, a reduction in the difference between the subject level of a sign of the disease and the normal level of the sign for the disease (e.g., the upper level of normal when the level must be reduced to reach a normal level, and the lower level of normal when the level must be increased to reach a normal level). In certain embodiments, the methods include a clinically relevant inhibition of expression of XDH, e.g., as demonstrated by a clinically relevant outcome after treatment of a subject with an agent to reduce the expression of XDH.

As used herein, "prevention" or "preventing," when used in reference to a disease, disorder, or condition, that would benefit from a reduction in expression of an XDH gene or production of XDH protein, i.e., a disease, disorder, or condition that would benefit from reduction of a chronically elevated uric acid level, refers to a reduction in the likelihood that a subject will develop a symptom associated with such a disease, disorder, or condition, e.g., a symptom of XDH gene expression, such as the presence of chronic elevated serum levels of uric acid, e.g., hyperuricemia, gout, NASH, NAFLD, metabolic disorder, or cardiovascular disease. The failure to develop a disease, disorder or condition, or the reduction in the development of a symptom or comorbidity associated with such a disease, disorder or condition (e.g., by at least about 10% on a clinically accepted scale for that disease or disorder), the exhibition of delayed symptoms or disease progression by days, weeks, months or years, or the reduction or maintenance of a serum uric acid level at 6.8 mg/dl or less in a subject prone to elevated serum uric acid is considered effective prevention. Prevention may require the administration of more than one dose.

As used herein, the term "xanthine dehydrogenase-associated disease" or "XDH-associated disease," is a disease or disorder that is caused by, or associated with XDH gene expression or XDH protein production, e.g., a disease, disorder, or condition associated with chronic elevated levels of serum uric acid. The term "XDH-associated disease" includes a disease, disorder, or condition that would benefit from a decrease in XDH gene expression, replication, or protein activity. Non-limiting examples of XDH-associated diseases include, for example, hyperuricemia, gout, NAFLD, NASH, metabolic disorder, insulin resistance, cardiovascular disease, type 2 diabetes, and conditions linked to oxidative stress e.g., chronic low grade inflammation; or other XDH-associated disease.

In certain embodiments, an XDH-associated disease is gout. In certain embodiments, an XDH-associated disease is NASH or NAFLD.

As used herein, "chronic renal insufficiency" or "chronic kidney disease" is a condition characterized by a gradual loss of kidney function over time. Chronic kidney disease is commonly caused by high blood pressure or diabetes, but

may result from other diseases or conditions such as lupus or may be caused by medications that have renal toxicity.

"Glomerulonephritis" includes a group of diseases that cause inflammation and damage to the kidney's filtering units. Inherited diseases, such as polycystic kidney disease, which causes large cysts to form in the kidneys and damage the surrounding tissue.

Congenital structural malformation of the kidneys, kidney stones or other obstructions (e.g., enlarged prostate) can result in chronic kidney disease.

Without being bound by mechanism, reduced kidney function can result in impaired renal clearance of uric acid. Kidney function is typically assessed by glomerular filtration rate (GFR) which is calculated based on blood creatinine level, age, body size, and gender. A lower GFR is indicative of lower kidney function. Kidney disease is typically diagnosed with a GFR of less than 60 for at least three months or a GFR of 60 or less with high protein in the urine is diagnostic of chronic kidney disease. However, a GFR of less than 90 is indicative of kidney damage with mild loss of kidney function.

"Therapeutically effective amount," as used herein, is intended to include the amount of an iRNA that, when administered to a patient for treating a subject having hyperuricemia, gout, NAFLD, NASH, metabolic disorder, insulin resistance, cardiovascular disease, type 2 diabetes, and conditions linked to oxidative stress, e.g., chronic low grade inflammation; or other XDH-associated disease, is sufficient to effect treatment of the disease (e.g., by diminishing, ameliorating or maintaining the existing disease or one or more symptoms of disease or its related comorbidities). The "therapeutically effective amount" may vary depending on the iRNA, how it is administered, the disease and its severity and the history, age, weight, family history, genetic makeup, stage of pathological processes mediated by XDH gene expression, the types of preceding or concomitant treatments, if any, and other individual characteristics of the patient to be treated. Treatment may require the administration of more than one dose.

As used herein, a therapeutically effective amount is sufficient to result in a clinically relevant lowering of chronic serum uric acid level in a subject and does not necessarily require the lowering of the chronic serum uric acid level to below 6.8 mg/dl. In certain embodiments, a therapeutically effective amount of an iRNA will lower the chronic uric acid of a subject to 6.8 mg/dl of uric acid or less, e.g., to 6 mg/dl or less, and optionally no lower than about 2 mg/dl. Studies of subjects with inherited disorders associated with profound, lifelong hypouricemia indicate that maintaining serum uric acid near or below 2 mg/dl is safe (Hershfeld, *Curr. Opin. Rheumatol.* 21:138-142, 2009).

"Prophylactically effective amount," as used herein, is intended to include the amount of an iRNA that, when administered to a subject who does not yet experience or display symptoms of hyperuricemia, gout, NAFLD, NASH, metabolic disorder, insulin resistance, cardiovascular disease, type 2 diabetes, and conditions linked to oxidative stress, e.g., chronic low grade inflammation; or other XDH-associated disease, but who may be predisposed to an XDH-associated disease, is sufficient to prevent or delay the development or progression of the disease or one or more symptoms of the disease for a clinically significant period of time, e.g., to lower a chronically elevated serum uric acid level, to prevent an increase in chronic serum uric acid level or to maintain a uric acid level below 6.8 mg/dl in a subject prone to elevated chronic serum uric acid level. The "prophylactically effective amount" may vary depending on the iRNA, how it is administered, the degree of risk of disease, and the history, age, weight, family history, genetic makeup, the types of preceding or concomitant treatments, if any, and other individual characteristics of the patient to be treated.

A "therapeutically-effective amount" or "prophylacticaly effective amount" also includes an amount of an iRNA that produces some desired local or systemic effect at a reasonable benefit/risk ratio applicable to any treatment. iRNAs employed in the methods of the present invention may be administered in a sufficient amount to produce a reasonable benefit/risk ratio applicable to such treatment. For example, in certain embodiments, treatment with the iRNAs of the invention will result in serum uric acid levels at 2-6.8 mg/dl, preferably 2-6 mg/dl in subjects. Maintenance of such uric acid levels will treat or prevent XDH-associated diseases.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human subjects and animal subjects without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The phrase "pharmaceutically-acceptable carrier" as used herein means a pharmaceutically-acceptable material, composition, or vehicle, such as a liquid or solid filler, diluent, excipient, manufacturing aid (e.g., lubricant, talc magnesium, calcium or zinc stearate, or steric acid), or solvent encapsulating material, involved in carrying or transporting the subject compound from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the subject being treated. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) lubricating agents, such as magnesium state, sodium lauryl sulfate and talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) pH buffered solutions; (21) polyesters, polycarbonates or polyanhydrides; (22) bulking agents, such as polypeptides and amino acids (23) serum component, such as serum albumin, HDL and LDL; and (22) other non-toxic compatible substances employed in pharmaceutical formulations.

The term "sample," as used herein, includes a collection of similar fluids, cells, or tissues isolated from a subject, as well as fluids, cells, or tissues present within a subject. Examples of biological fluids include blood, serum and serosal fluids, plasma, cerebrospinal fluid, ocular fluids, lymph, urine, saliva, and the like. Tissue samples may include samples from tissues, organs, or localized regions. For example, samples may be derived from particular organs, parts of organs, or fluids or cells within those organs. In certain embodiments, samples may be derived from the liver (e.g., whole liver or certain segments of liver or certain types of cells in the liver, such as, e.g., hepatocytes). A

"sample derived from a subject" can refer to blood drawn from the subject or plasma derived therefrom.

I. iRNAs of the Invention

The present invention provides iRNAs which inhibit the expression of an XDH gene. In preferred embodiments, the iRNA is a double stranded ribonucleic acid (dsRNA) molecule for inhibiting the expression of an XDH gene in a cell, such as a cell within a subject, e.g., a mammal, such as a human having an XDH-associated disease, e.g., hyperuricemia, gout. The dsRNA agent includes an antisense strand having a region of complementarity which is complementary to at least a part of an mRNA formed in the expression of an XDH gene. The region of complementarity is about 30 nucleotides or less in length (e.g., about 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, or 18 nucleotides or less in length). Upon contact with a cell expressing the XDH gene, the iRNA inhibits the expression of the XDH gene (e.g., a human, a primate, a non-primate, or a bird XDH gene) by at least about 20% as assayed by, for example, a PCR or branched DNA (bDNA)-based method, or by a protein-based method, such as by immunofluorescence analysis, using, for example, western blotting or flow cytometric techniques. In preferred embodiments, inhibition of expression is determined by the qPCR method provided in the examples. For in vitro assessment of activity, percent inhibition is determined using the methods provided in Example 2 at a single dose at a 10 nM duplex final concentration. For in vivo studies, the level after treatment can be compared to, for example, an appropriate historical control or a pooled population sample control to determine the level of reduction, e.g., when a baseline value is not available for the subject.

A dsRNA agent includes two RNA strands that are complementary and hybridize to form a duplex structure under conditions in which the dsRNA will be used. One strand of a dsRNA (the antisense strand) includes a region of complementarity that is substantially complementary, and generally fully complementary, to a target sequence. The target sequence can be derived from the sequence of an mRNA formed during the expression of an XDH gene. The other strand (the sense strand) includes a region that is complementary to the antisense strand, such that the two strands hybridize and form a duplex structure when combined under suitable conditions. As described elsewhere herein and as known in the art, the complementary sequences of a dsRNA can also be contained as self-complementary regions of a single nucleic acid molecule, as opposed to being on separate oligonucleotides.

Generally, the duplex structure is 15 to 30 base pairs in length, e.g., 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

Similarly, the region of complementarity to the target sequence may be 15 to 30 nucleotides in length, e.g., 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 nucleotides in length. Ranges and lengths intermediate to the above recited ranges and lengths are also contemplated to be part of the invention.

In some embodiments, the dsRNA is about 15 to 23 nucleotides in length, or about 25 to 30 nucleotides in length. In general, the dsRNA is long enough to serve as a substrate for the Dicer enzyme. For example, it is well-known in the art that dsRNAs longer than about 21-23 nucleotides in length may serve as substrates for Dicer. As the ordinarily skilled person will also recognize, the region of an RNA targeted for cleavage will most often be part of a larger RNA molecule, often an mRNA molecule. Where relevant, a "part" of an mRNA target is a contiguous sequence of an mRNA target of sufficient length to allow it to be a substrate for RNAi-directed cleavage (i.e., cleavage through a RISC pathway).

One of skill in the art will also recognize that the duplex region is a primary functional portion of a dsRNA, e.g., a duplex region of about 9 to about 36 base pairs, e.g., about 10-36, 11-36, 12-36, 13-36, 14-36, 15-36, 9-35, 10-35, 11-35, 12-35, 13-35, 14-35, 15-35, 9-34, 10-34, 11-34, 12-34, 13-34, 14-34, 15-34, 9-33, 10-33, 11-33, 12-33, 13-33, 14-33, 15-33, 9-32, 10-32, 11-32, 12-32, 13-32, 14-32, 15-32, 9-31, 10-31, 11-31, 12-31, 13-32, 14-31, 15-31, 15-30, 15-29, 15-28, 15-27, 15-26, 15-25, 15-24, 15-23, 15-22, 15-21, 15-20, 15-19, 15-18, 15-17, 18-30, 18-29, 18-28, 18-27, 18-26, 18-25, 18-24, 18-23, 18-22, 18-21, 18-20, 19-30, 19-29, 19-28, 19-27, 19-26, 19-25, 19-24, 19-23, 19-22, 19-21, 19-20, 20-30, 20-29, 20-28, 20-27, 20-26, 20-25, 20-24, 20-23, 20-22, 20-21, 21-30, 21-29, 21-28, 21-27, 21-26, 21-25, 21-24, 21-23, or 21-22 base pairs. Thus, in one embodiment, to the extent that it becomes processed to a functional duplex, of e.g., 15-30 base pairs, that targets a desired RNA for cleavage, an RNA molecule or complex of RNA molecules having a duplex region greater than 30 base pairs is a dsRNA. Thus, an ordinarily skilled artisan will recognize that in one embodiment, a miRNA is a dsRNA. In another embodiment, a dsRNA is not a naturally occurring miRNA. In another embodiment, an iRNA agent useful to target XDH gene expression is not generated in the target cell by cleavage of a larger dsRNA.

A dsRNA as described herein can further include one or more single-stranded nucleotide overhangs e.g., 1-4, 2-4, 1-3, 2-3, 1, 2, 3, or 4 nucleotides. dsRNAs having at least one nucleotide overhang can have superior inhibitory properties relative to their blunt-ended counterparts. A nucleotide overhang can comprise or consist of a nucleotide/nucleoside analog, including a deoxynucleotide/nucleoside. The overhang(s) can be on the sense strand, the antisense strand, or any combination thereof. Furthermore, the nucleotide(s) of an overhang can be present on the 5'-end, 3'-end, or both ends of an antisense or sense strand of a dsRNA.

A dsRNA can be synthesized by standard methods known in the art as further discussed below, e.g., by use of an automated DNA synthesizer, such as are commercially available from, for example, Biosearch, Applied Biosystems, Inc.

Double stranded RNAi compounds of the invention may be prepared using a two-step procedure. First, the individual strands of the double stranded RNA molecule are prepared separately. Then, the component strands are annealed. The individual strands of the siRNA compound can be prepared using solution-phase or solid-phase organic synthesis or both. Organic synthesis offers the advantage that the oligo-

nucleotide strands comprising unnatural or modified nucleotides can be easily prepared. Similarly, single-stranded oligonucleotides of the invention can be prepared using solution-phase or solid-phase organic synthesis or both.

In an aspect, a dsRNA of the invention includes at least two nucleotide sequences, a sense sequence and an antisense sequence. The sense strand is selected from the group of sequences provided in any one of Tables 3, 4, 6 and 7, and the corresponding antisense strand of the sense strand is selected from the group of sequences of any one of Tables 3, 4, 6 and 7. In this aspect, one of the two sequences is complementary to the other of the two sequences, with one of the sequences being substantially complementary to a sequence of an mRNA generated in the expression of an XDH gene. As such, in this aspect, a dsRNA will include two oligonucleotides, where one oligonucleotide is described as the sense strand in any one of Tables 3, 4, 6 and 7, and the second oligonucleotide is described as the corresponding antisense strand of the sense strand in any one of Tables 3, 4, 6 and 7. In certain embodiments, the substantially complementary sequences of the dsRNA are contained on separate oligonucleotides. In other embodiments, the substantially complementary sequences of the dsRNA are contained on a single oligonucleotide.

It will be understood that, although some of the sequences in Tables 3, 4, 6 and 7 are described as modified and/or conjugated sequences, the RNA of the iRNA of the invention e.g., a dsRNA of the invention, may comprise any one of the sequences set forth in Tables 3, 4, 6 and 7 that is unmodified, un-conjugated, and/or modified and/or conjugated differently than described therein.

The skilled person is well aware that dsRNAs having a duplex structure of about 20 to 23 base pairs, e.g., 21, base pairs have been hailed as particularly effective in inducing RNA interference (Elbashir et al., *EMBO* 2001, 20:6877-6888). However, others have found that shorter or longer RNA duplex structures can also be effective (Chu and Rana (2007) *RNA* 14:1714-1719; Kim et al. (2005) *Nat Biotech* 23:222-226). In the embodiments described above, by virtue of the nature of the oligonucleotide sequences provided in any one of Tables 3, 4, 6 and 7, dsRNAs described herein can include at least one strand of a length of minimally 21 nucleotides. It can be reasonably expected that shorter duplexes having one of the sequences of Tables 3, 4, 6 and 7 minus only a few nucleotides on one or both ends can be similarly effective as compared to the dsRNAs described above. Hence, dsRNAs having a sequence of at least 15, 16, 17, 18, 19, 20, or more contiguous nucleotides derived from one of the sequences of Tables 3, 4, 6 and 7, and differing in their ability to inhibit the expression of an XDH gene by not more than about 5, 10, 15, 20, 25, or 30% inhibition from a dsRNA comprising the full sequence, are contemplated to be within the scope of the present invention.

In addition, the RNAs provided in Tables 3, 4, 6 and 7 identify a site(s) in an XDH transcript that is susceptible to RISC-mediated cleavage. As such, the present invention further features iRNAs that target within one of these sites. As used herein, an iRNA is said to target within a particular site of an RNA transcript if the iRNA promotes cleavage of the transcript anywhere within that particular site. Such an iRNA will generally include at least about 15 contiguous nucleotides from one of the sequences provided in Tables 3, 4, 6 and 7 coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in an XDH gene.

While a target sequence is generally about 15-30 nucleotides in length, there is wide variation in the suitability of particular sequences in this range for directing cleavage of any given target RNA. Various software packages and the guidelines set out herein provide guidance for the identification of optimal target sequences for any given gene target, but an empirical approach can also be taken in which a "window" or "mask" of a given size (as a non-limiting example, 21 nucleotides) is literally or figuratively (including, e.g., in silico) placed on the target RNA sequence to identify sequences in the size range that can serve as target sequences. By moving the sequence "window" progressively one nucleotide upstream or downstream of an initial target sequence location, the next potential target sequence can be identified, until the complete set of possible sequences is identified for any given target size selected. This process, coupled with systematic synthesis and testing of the identified sequences (using assays as described herein or as known in the art or provided herein) to identify those sequences that perform optimally can identify those RNA sequences that, when targeted with an iRNA agent, mediate the best inhibition of target gene expression. Thus, while the sequences identified, for example, in Tables 3, 4, 6 and 7 represent effective target sequences, it is contemplated that further optimization of inhibition efficiency can be achieved by progressively "walking the window" one nucleotide upstream or downstream of the given sequences to identify sequences with equal or better inhibition characteristics.

Further, it is contemplated that for any sequence identified, e.g., in Tables 3, 4, 6 and 7, further optimization could be achieved by systematically either adding or removing nucleotides to generate longer or shorter sequences and testing those sequences generated by walking a window of the longer or shorter size up or down the target RNA from that point. Again, coupling this approach to generating new candidate targets with testing for effectiveness of iRNAs based on those target sequences in an inhibition assay as known in the art or as described herein can lead to further improvements in the efficiency of inhibition. Further still, such optimized sequences can be adjusted by, e.g., the introduction of modified nucleotides as described herein or as known in the art, addition or changes in overhang, or other modifications as known in the art or discussed herein to further optimize the molecule (e.g., increasing serum stability or circulating half-life, increasing thermal stability, enhancing transmembrane delivery, targeting to a particular location or cell type, increasing interaction with silencing pathway enzymes, increasing release from endosomes) as an expression inhibitor.

An iRNA as described herein can contain one or more mismatches to the target sequence. In one embodiment, an iRNA as described herein contains no more than 3 mismatches. If the antisense strand of the iRNA contains mismatches to a target sequence, it is preferable that the area of mismatch is not located in the center of the region of complementarity. If the antisense strand of the iRNA contains mismatches to the target sequence, it is preferable that the mismatch be restricted to be within the last 5 nucleotides from either the 5'- or 3'-end of the region of complementarity. For example, for a 23 nucleotide iRNA agent the strand which is complementary to a region of an XDH gene, generally does not contain any mismatch within the central 13 nucleotides. The methods described herein or methods known in the art can be used to determine whether an iRNA containing a mismatch to a target sequence is effective in inhibiting the expression of an XDH gene. Consideration of the efficacy of iRNAs with mismatches in inhibiting expression of an XDH gene is important, especially if the particular

region of complementarity in an XDH gene is known to have polymorphic sequence variation within the population.

II. Modified iRNAs of the Invention

In certain embodiments, the RNA of the iRNA of the invention e.g., a dsRNA, is un-modified, and does not comprise, e.g., chemical modifications or conjugations known in the art and described herein. In other embodiments, the RNA of an iRNA of the invention, e.g., a dsRNA, is chemically modified to enhance stability or other beneficial characteristics. In certain embodiments of the invention, substantially all of the nucleotides of an iRNA of the invention are modified. In other embodiments of the invention, all of the nucleotides of an iRNA or substantially all of the nucleotides of an iRNA are modified, i.e., not more than 5, 4, 3, 2, or 1 unmodified nucleotides are present in a strand of the iRNA.

The nucleic acids featured in the invention can be synthesized or modified by methods well established in the art, such as those described in "Current protocols in nucleic acid chemistry," Beaucage, S. L. et al. (Edrs.), John Wiley & Sons, Inc., New York, N.Y., USA, which is hereby incorporated herein by reference. Modifications include, for example, end modifications, e.g., 5'-end modifications (phosphorylation, conjugation, inverted linkages) or 3'-end modifications (conjugation, DNA nucleotides, inverted linkages, etc.); base modifications, e.g., replacement with stabilizing bases, destabilizing bases, or bases that base pair with an expanded repertoire of partners, removal of bases (abasic nucleotides), or conjugated bases; sugar modifications (e.g., at the 2'-position or 4'-position) or replacement of the sugar; or backbone modifications, including modification or replacement of the phosphodiester linkages. Specific examples of iRNA compounds useful in the embodiments described herein include, but are not limited to RNAs containing modified backbones or no natural internucleoside linkages. RNAs having modified backbones include, among others, those that do not have a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced in the art, modified RNAs that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides. In some embodiments, a modified iRNA will have a phosphorus atom in its internucleoside backbone.

Modified RNA backbones include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5'-linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included.

Representative US patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S. Pat. Nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,195; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,316; 5,550,111; 5,563,253; 5,571,799; 5,587,361; 5,625,050; 6,028,188; 6,124,445; 6,160,109; 6,169,170; 6,172,209; 6,239,265; 6,277,603; 6,326,199; 6,346,614; 6,444,423; 6,531,590; 6,534,639; 6,608,035; 6,683,167; 6,858,715; 6,867,294; 6,878,805; 7,015,315; 7,041,816; 7,273,933; 7,321,029; and U.S. Pat. No. RE39464, the entire contents of each of which are hereby incorporated herein by reference.

Modified RNA backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatoms and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S, and CH_2 component parts.

Representative US patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S. Pat. Nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,64,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and 5,677,439, the entire contents of each of which are hereby incorporated herein by reference.

Suitable RNA mimetics are contemplated for use in iRNAs provided herein, in which both the sugar and the internucleoside linkage, i.e., the backbone, of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound in which an RNA mimetic that has been shown to have excellent hybridization properties is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar backbone of an RNA is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative US patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Pat. Nos. 5,539,082; 5,714,331; and 5,719,262, the entire contents of each of which are hereby incorporated herein by reference. Additional PNA compounds suitable for use in the iRNAs of the invention are described in, for example, in Nielsen et al., *Science,* 1991, 254, 1497-1500.

Some embodiments featured in the invention include RNAs with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular —CH_2—NH—CH_2—, —CH_2—N(CH_3)—O—CH_2— [known as a methylene (methylimino) or MMI backbone], —CH_2—O—N(CH_3)—CH_2—, —CH_2—N(CH_3)—N(CH_3)—CH_2— and —N(CH_3)—CH_2—CH_2— [wherein the native phosphodiester backbone is represented as —O—P—O—CH_2—] of the above-referenced U.S. Pat. No. 5,489,677, and the amide backbones of the above-referenced U.S. Pat. No. 5,602,240. In some embodiments, the RNAs featured herein have morpholino backbone structures of the above-referenced U.S. Pat. No. 5,034,506.

Modified RNAs can also contain one or more substituted sugar moieties. The iRNAs, e.g., dsRNAs, featured herein can include one of the following at the 2'-position: OH; F; O—, S—, or N-alkyl; O—, S—, or N-alkenyl; O—, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl can be substituted or unsubstituted C_1 to C_{10}

alkyl or C_2 to C_{10} alkenyl and alkynyl. Exemplary suitable modifications include $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_nOCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nONH_2$, and $O(CH_2)_nON[(CH_2)_nCH_3)]_2$, where n and m are from 1 to about 10. In other embodiments, dsRNAs include one of the following at the 2' position: C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an iRNA, or a group for improving the pharmacodynamic properties of an iRNA, and other substituents having similar properties. In some embodiments, the modification includes a 2'-methoxyethoxy (2'-O—$CH_2CH_2OCH_3$, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin et al., *Helv. Chim. Acta,* 1995, 78:486-504) i.e., an alkoxy-alkoxy group. Another exemplary modification is 2'-dimethylaminooxyethoxy, i.e., a $O(CH_2)_2ON(CH_3)_2$ group, also known as 2'-DMAOE, as described in examples herein below, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), i.e., 2'-O—CH_2—O—CH_2—$N(CH_2)_2$. Further exemplary modifications include: 5'-Me-2'-F nucleotides, 5'-Me-2'-OMe nucleotides, 5'-Me-2'-deoxynucleotides, (both R and S isomers in these three families); 2'-alkoxyalkyl; and 2'-NMA (N-methylacetamide).

Other modifications include 2'-methoxy (2'-OCH_3), 2'-aminopropoxy (2'-$OCH_2CH_2CH_2NH_2$) and 2'-fluoro (2'-F) Similar modifications can also be made at other positions on the RNA of an iRNA, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked dsRNAs and the 5' position of 5' terminal nucleotide. iRNAs can also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative US patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S. Pat. Nos. 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, certain of which are commonly owned with the instant application. The entire contents of each of the foregoing are hereby incorporated herein by reference.

An iRNA can also include nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C), and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as deoxy-thymine (dT), 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl anal other 8-substituted adenines and guanines, 5-halo, particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-daazaadenine and 3-deazaguanine and 3-deazaadenine. Further nucleobases include those disclosed in U.S. Pat. No. 3,687,808, those disclosed in Modified Nucleosides in Biochemistry, Biotechnology and Medicine, Herdewijn, P. ed. Wiley-VCH, 2008; those disclosed in The Concise Encyclopedia Of Polymer Science And Engineering, pages 858-859, Kroschwitz, J. L, ed. John Wiley & Sons, 1990, these disclosed by Englisch et al., Angewandte Chemie, International Edition, 1991, 30, 613, and those disclosed by Sanghvi, Y S., Chapter 15, dsRNA Research and Applications, pages 289-302, Crooke, S. T. and Lebleu, B., Ed., CRC Press, 1993. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds featured in the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and 0-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2° C. (Sanghvi, Y. S., Crooke, S. T. and Lebleu, B., Eds., dsRNA Research and Applications, CRC Press, Boca Raton, 1993, pp. 276-278) and are exemplary base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

Representative US patents that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include, but are not limited to, the above noted U.S. Pat. Nos. 3,687,808, 4,845,205; 5,130,30; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; 5,681,941; 5,750,692; 6,015,886; 6,147,200; 6,166,197; 6,222,025; 6,235,887; 6,380,368; 6,528,640; 6,639,062; 6,617,438; 7,045,610; 7,427,672; and 7,495,088, the entire contents of each of which are hereby incorporated herein by reference.

The RNA of an iRNA can also be modified to include one or more locked nucleic acids (LNA). A locked nucleic acid is a nucleotide having a modified ribose moiety in which the ribose moiety comprises an extra bridge connecting the 2' and 4' carbons. This structure effectively "locks" the ribose in the 3'-endo structural conformation. The addition of locked nucleic acids to siRNAs has been shown to increase siRNA stability in serum, and to reduce off-target effects (Elmen, J. et al., (2005) *Nucleic Acids Research* 33(1):439-447; Mook, O R. et al., (2007) *Mol Canc Ther* 6)(3):833-843; Grunweller, A. et al., (2003) *Nucleic Acids Research* 31(12):3185-3193).

In some embodiments, the iRNA of the invention comprises one or more monomers that are UNA (unlocked nucleic acid) nucleotides. UNA is unlocked acyclic nucleic acid, wherein any of the bonds of the sugar has been removed, forming an unlocked "sugar" residue. In one example, UNA also encompasses monomer with bonds between C1'—C4' have been removed (i.e. the covalent carbon-oxygen-carbon bond between the C1' and C4' carbons). In another example, the C2'—C3' bond (i.e. the covalent carbon-carbon bond between the C2' and C3' carbons) of the sugar has been removed (see *Nuc. Acids Symp. Series,* 52, 133-134 (2008) and Fluiter et al., *Mol. Biosyst.,* 2009, 10, 1039 hereby incorporated by reference).

The RNA of an iRNA can also be modified to include one or more bicyclic sugar moities. A "bicyclic sugar" is a furanosyl ring modified by the bridging of two atoms. A "bicyclic nucleoside" ("BNA") is a nucleoside having a sugar moiety comprising a bridge connecting two carbon atoms of the sugar ring, thereby forming a bicyclic ring system. In certain embodiments, the bridge connects the 4'-carbon and the 2'-carbon of the sugar ring. Thus, in some embodiments an agent of the invention may include one or more locked nucleic acids (LNA). A locked nucleic acid is a nucleotide having a modified ribose moiety in which the

ribose moiety comprises an extra bridge connecting the 2' and 4' carbons. In other words, an LNA is a nucleotide comprising a bicyclic sugar moiety comprising a 4'-CH_2—O-2' bridge. This structure effectively "locks" the ribose in the 3'-endo structural conformation. The addition of locked nucleic acids to siRNAs has been shown to increase siRNA stability in serum, and to reduce off-target effects (Elmen, J. et al., (2005) *Nucleic Acids Research* 33(1):439-447; Mook, O R. et al., (2007) *Mol Canc Ther* 6β): 833-843; Grunweller, A. et al., (2003) *Nucleic Acids Research* 31(12):3185-3193). Examples of bicyclic nucleosides for use in the polynucleotides of the invention include without limitation nucleosides comprising a bridge between the 4' and the 2' ribosyl ring atoms. In certain embodiments, the antisense polynucleotide agents of the invention include one or more bicyclic nucleosides comprising a 4' to 2' bridge. Examples of such 4' to 2' bridged bicyclic nucleosides, include but are not limited to 4'-(CH_2)—O-2' (LNA); 4'-(CH_2)—S-2; 4'—(CH_2)$_2$—O-2' (ENA); 4'-CH(CH_3)—O-2' (also referred to as "constrained ethyl" or "cEt") and 4'-CH(CH_2OCH_3)—O-2' (and analogs thereof; see, e.g., U.S. Pat. No. 7,399,845); 4'-C(CH_3)(CH_3)—O-2' (and analogs thereof; see e.g., U.S. Pat. No. 8,278,283); 4'-CH_2—N(OCH_3)-2' (and analogs thereof; see e.g., U.S. Pat. No. 8,278,425); 4'-CH_2—O—N(CH_3)-2' (see, e.g., US Patent Publication No. 2004/0171570); 4'-CH_2—N(R)—O-2', wherein R is H, C1-C12 alkyl, or a protecting group (see, e.g., U.S. Pat. No. 7,427,672); 4'-CH_2—C(H)(CH_3)-2' (see, e.g., Chattopadhyaya et al., *J. Org. Chem.,* 2009, 74, 118-134); and 4'-CH_2—C(=CH_2)-2' (and analogs thereof; see, e.g., U.S. Pat. No. 8,278,426). The entire contents of each of the foregoing are hereby incorporated herein by reference.

Additional representative US patents and US patent Publications that teach the preparation of locked nucleic acid nucleotides include, but are not limited to, the following: U.S. Pat. Nos. 6,268,490; 6,525,191; 6,670,461; 6,770,748; 6,794,499; 6,998,484; 7,053,207; 7,034,133; 7,084,125; 7,399,845; 7,427,672; 7,569,686; 7,741,457; 8,022,193; 8,030,467; 8,278,425; 8,278,426; 8,278,283; US 2008/0039618; and US 2009/0012281, the entire contents of each of which are hereby incorporated herein by reference.

Any of the foregoing bicyclic nucleosides can be prepared having one or more stereochemical sugar configurations including for example α-L-ribofuranose and β-D-ribofuranose (see WO 99/14226).

The RNA of an iRNA can also be modified to include one or more constrained ethyl nucleotides. As used herein, a "constrained ethyl nucleotide" or "cEt" is a locked nucleic acid comprising a bicyclic sugar moiety comprising a 4'-CH(CH_3)—O-2' bridge. In one embodiment, a constrained ethyl nucleotide is in the S conformation referred to herein as "S-cEt."

An iRNA of the invention may also include one or more "conformationally restricted nucleotides" ("CRN"). CRN are nucleotide analogs with a linker connecting the C2' and C4' carbons of ribose or the C3 and —C5' carbons of ribose. CRN lock the ribose ring into a stable conformation and increase the hybridization affinity to mRNA. The linker is of sufficient length to place the oxygen in an optimal position for stability and affinity resulting in less ribose ring puckering.

Representative publications that teach the preparation of certain of the above noted CRN include, but are not limited to, US Patent Publication No. 2013/0190383; and PCT publication WO 2013/036868, the entire contents of each of which are hereby incorporated herein by reference.

In some embodiments, the iRNA of the invention comprises one or more monomers that are UNA (unlocked nucleic acid) nucleotides. UNA is unlocked acyclic nucleic acid, wherein any of the bonds of the sugar has been removed, forming an unlocked "sugar" residue. In one example, UNA also encompasses monomer with bonds between C1'—C4' have been removed (i.e. the covalent carbon-oxygen-carbon bond between the C1' and C4' carbons). In another example, the C2'—C3' bond (i.e. the covalent carbon-carbon bond between the C2' and C3' carbons) of the sugar has been removed (see *Nuc. Acids Symp. Series,* 52, 133-134 (2008) and Fluiter et al., *Mol. Biosyst.,* 2009, 10, 1039 hereby incorporated by reference).

Representative US publications that teach the preparation of UNA include, but are not limited to, U.S. Pat. No. 8,314,227; and US Patent Publication Nos. 2013/0096289; 2013/0011922; and 2011/0313020, the entire contents of each of which are hereby incorporated herein by reference.

Potentially stabilizing modifications to the ends of RNA molecules can include N-(acetylaminocaproyl)-4-hydroxyprolinol (Hyp-C6-NHAc), N-(caproyl-4-hydroxyprolinol (Hyp-C6), N-(acetyl-4-hydroxyprolinol (Hyp-NHAc), thymidine-2'-0-deoxythymidine (ether), N-(aminocaproyl)-4-hydroxyprolinol (Hyp-C6-amino), 2-docosanoyl-uridine-3"-phosphate, inverted base dT(idT) and others. Disclosure of this modification can be found in PCT Publication No. WO 2011/005861.

Other modifications of the nucleotides of an iRNA of the invention include a 5' phosphate or 5' phosphate mimic, e.g., a 5'-terminal phosphate or phosphate mimic on the antisense strand of an iRNA. Suitable phosphate mimics are disclosed in, for example US Patent Publication No. 2012/0157511, the entire contents of which are incorporated herein by reference.

A. Modified iRNAs Comprising Motifs of the Invention

In certain aspects of the invention, the double stranded RNAi agents of the invention include agents with chemical modifications as disclosed, for example, in U.S. Patent Publication No. 2014/0315835 and PCT Publication No. WO 2013/075035, the entire contents of each of which are incorporated herein by reference. WO 2013/075035 and U.S. 2014/0315835 provide motifs of three identical modifications on three consecutive nucleotides into a sense strand or antisense strand of a dsRNAi agent, particularly at or near the cleavage site. In some embodiments, the sense strand and antisense strand of the dsRNAi agent may otherwise be completely modified. The introduction of these motifs interrupts the modification pattern, if present, of the sense or antisense strand. The dsRNAi agent may be optionally conjugated with a GalNAc derivative ligand, for instance on the sense strand.

More specifically, when the sense strand and antisense strand of the double stranded RNAi agent are completely modified to have one or more motifs of three identical modifications on three consecutive nucleotides at or near the cleavage site of at least one strand of a dsRNAi agent, the gene silencing activity of the dsRNAi agent was observed.

Accordingly, the invention provides double stranded RNAi agents capable of inhibiting the expression of a target gene (i.e., XDH gene) in vivo. The RNAi agent comprises a sense strand and an antisense strand. Each strand of the RNAi agent may be, independently, 12-30 nucleotides in length. For example, each strand may independently be 14-30 nucleotides in length, 17-30 nucleotides in length, 25-30 nucleotides in length, 27-30 nucleotides in length, 17-23 nucleotides in length, 17-21 nucleotides in length, 17-19 nucleotides in length, 19-25 nucleotides in length,

19-23 nucleotides in length, 19-21 nucleotides in length, 21-25 nucleotides in length, or 21-23 nucleotides in length.

The sense strand and antisense strand typically form a duplex double stranded RNA ("dsRNA"), also referred to herein as "dsRNAi agent." The duplex region of a dsRNAi agent may be 12-30 nucleotide pairs in length. For example, the duplex region can be 14-30 nucleotide pairs in length, 17-30 nucleotide pairs in length, 27-30 nucleotide pairs in length, 17-23 nucleotide pairs in length, 17-21 nucleotide pairs in length, 17-19 nucleotide pairs in length, 19-25 nucleotide pairs in length, 19-23 nucleotide pairs in length, 19-21 nucleotide pairs in length, 21-25 nucleotide pairs in length, or 21-23 nucleotide pairs in length. In another example, the duplex region is selected from 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, and 30 nucleotides in length.

In certain embodiments, the dsRNAi agent may contain one or more overhang regions or capping groups at the 3'-end, 5'-end, or both ends of one or both strands. The overhang can be, independently, 1-6 nucleotides in length, for instance 2-6 nucleotides in length, 1-5 nucleotides in length, 2-5 nucleotides in length, 1-4 nucleotides in length, 2-4 nucleotides in length, 1-3 nucleotides in length, 2-3 nucleotides in length, or 1-2 nucleotides in length. In certain embodiments, the overhang regions can include extended overhang regions as provided above. The overhangs can be the result of one strand being longer than the other, or the result of two strands of the same length being staggered. The overhang can form a mismatch with the target mRNA or it can be complementary to the gene sequences being targeted or can be another sequence. The first and second strands can also be joined, e.g., by additional bases to form a hairpin, or by other non-base linkers.

In certain embodiments, the nucleotides in the overhang region of the dsRNAi agent can each independently be a modified or unmodified nucleotide including, but no limited to 2'-sugar modified, such as, 2'-F, 2'-O-methyl, thymidine (T), 2'-O-methoxyethyl-5-methyluridine (Teo), 2'-O-methoxyethyladenosine (Aeo), 2'-O-methoxyethyl-5-methylcytidine (m5Ceo), and any combinations thereof. For example, TT can be an overhang sequence for either end on either strand. The overhang can form a mismatch with the target mRNA or it can be complementary to the gene sequences being targeted or can be another sequence.

The 5'- or 3'-overhangs at the sense strand, antisense strand, or both strands of the dsRNAi agent may be phosphorylated. In some embodiments, the overhang region(s) contains two nucleotides having a phosphorothioate between the two nucleotides, where the two nucleotides can be the same or different. In some embodiments, the overhang is present at the 3'-end of the sense strand, antisense strand, or both strands. In some embodiments, this 3'-overhang is present in the antisense strand. In some embodiments, this 3'-overhang is present in the sense strand.

The dsRNAi agent may contain only a single overhang, which can strengthen the interference activity of the RNAi, without affecting its overall stability. For example, the single-stranded overhang may be located at the 3'-end of the sense strand or, alternatively, at the 3'-end of the antisense strand. The RNAi may also have a blunt end, located at the 5'-end of the antisense strand (or the 3'-end of the sense strand) or vice versa. Generally, the antisense strand of the dsRNAi agent has a nucleotide overhang at the 3'-end, and the 5'-end is blunt. While not wishing to be bound by theory, the asymmetric blunt end at the 5'-end of the antisense strand and 3'-end overhang of the antisense strand favor the guide strand loading into RISC process.

In certain embodiments, the dsRNAi agent is a double ended bluntmer of 19 nucleotides in length, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 7, 8, 9 from the 5' end. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, 13 from the 5' end.

In other embodiments, the dsRNAi agent is a double ended bluntmer of 20 nucleotides in length, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 8, 9, 10 from the 5' end. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, 13 from the 5' end.

In yet other embodiments, the dsRNAi agent is a double ended bluntmer of 21 nucleotides in length, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 9, 10, 11 from the 5' end. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, 13 from the 5' end.

In certain embodiments, the dsRNAi agent comprises a 21 nucleotide sense strand and a 23 nucleotide antisense strand, wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides at positions 9, 10, 11 from the 5' end; the antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at positions 11, 12, 13 from the 5' end, wherein one end of the RNAi agent is blunt, while the other end comprises a 2 nucleotide overhang. Preferably, the 2 nucleotide overhang is at the 3'-end of the antisense strand.

When the 2 nucleotide overhang is at the 3'-end of the antisense strand, there may be two phosphorothioate internucleotide linkages between the terminal three nucleotides, wherein two of the three nucleotides are the overhang nucleotides, and the third nucleotide is a paired nucleotide next to the overhang nucleotide. In one embodiment, the RNAi agent additionally has two phosphorothioate internucleotide linkages between the terminal three nucleotides at both the 5'-end of the sense strand and at the 5'-end of the antisense strand. In certain embodiments, every nucleotide in the sense strand and the antisense strand of the dsRNAi agent, including the nucleotides that are part of the motifs are modified nucleotides. In certain embodiments each residue is independently modified with a 2'-O-methyl or 3'-fluoro, e.g., in an alternating motif. Optionally, the dsRNAi agent further comprises a ligand (preferably GalNAc, e.g., $GalNAc_3$).

In certain embodiments, the dsRNAi agent comprises a sense and an antisense strand, wherein the sense strand is 25-30 nucleotide residues in length, wherein starting from the 5' terminal nucleotide (position 1) positions 1 to 23 of the first strand comprise at least 8 ribonucleotides; the antisense strand is 36-66 nucleotide residues in length and, starting from the 3' terminal nucleotide, comprises at least 8 ribonucleotides in the positions paired with positions 1-23 of sense strand to form a duplex; wherein at least the 3 ' terminal nucleotide of antisense strand is unpaired with sense strand, and up to 6 consecutive 3' terminal nucleotides are unpaired with sense strand, thereby forming a 3' single stranded overhang of 1-6 nucleotides; wherein the 5' terminus of antisense strand comprises from 10-30 consecutive nucleotides which are unpaired with sense strand, thereby forming a 10-30 nucleotide single stranded 5' overhang; wherein at least the sense strand 5' terminal and 3' terminal nucleotides are base paired with nucleotides of antisense

strand when sense and antisense strands are aligned for maximum complementarity, thereby forming a substantially duplexed region between sense and antisense strands; and antisense strand is sufficiently complementary to a target RNA along at least 19 ribonucleotides of antisense strand length to reduce target gene expression when the double stranded nucleic acid is introduced into a mammalian cell; and wherein the sense strand contains at least one motif of three 2'-F modifications on three consecutive nucleotides, where at least one of the motifs occurs at or near the cleavage site. The antisense strand contains at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at or near the cleavage site.

In certain embodiments, the dsRNAi agent comprises sense and antisense strands, wherein the dsRNAi agent comprises a first strand having a length which is at least 25 and at most 29 nucleotides and a second strand having a length which is at most 30 nucleotides with at least one motif of three 2'-O-methyl modifications on three consecutive nucleotides at position 11, 12, 13 from the 5' end;

wherein the 3' end of the first strand and the 5' end of the second strand form a blunt end and the second strand is 1-4 nucleotides longer at its 3' end than the first strand, wherein the duplex region region which is at least 25 nucleotides in length, and the second strand is sufficiently complementary to a target mRNA along at least 19 nucleotide of the second strand length to reduce target gene expression when the RNAi agent is introduced into a mammalian cell, and wherein Dicer cleavage of the dsRNAi agent preferentially results in an siRNA comprising the 3'-end of the second strand, thereby reducing expression of the target gene in the mammal. Optionally, the dsRNAi agent further comprises a ligand.

In certain embodiments, the sense strand of the dsRNAi agent contains at least one motif of three identical modifications on three consecutive nucleotides, where one of the motifs occurs at the cleavage site in the sense strand.

In certain embodiments, the antisense strand of the dsRNAi agent can also contain at least one motif of three identical modifications on three consecutive nucleotides, where one of the motifs occurs at or near the cleavage site in the antisense strand.

For a dsRNAi agent having a duplex region of 17-23 nucleotides in length, the cleavage site of the antisense strand is typically around the 10, 11, and 12 positions from the 5'-end. Thus the motifs of three identical modifications may occur at the 9, 10, 11 positions; the 10, 11, 12 positions; the 11, 12, 13 positions; the 12, 13, 14 positions; or the 13, 14, 15 positions of the antisense strand, the count starting from the first nucleotide from the 5'-end of the antisense strand, or, the count starting from the first paired nucleotide within the duplex region from the 5'-end of the antisense strand. The cleavage site in the antisense strand may also change according to the length of the duplex region of the dsRNAi agent from the 5'-end.

The sense strand of the dsRNAi agent may contain at least one motif of three identical modifications on three consecutive nucleotides at the cleavage site of the strand; and the antisense strand may have at least one motif of three identical modifications on three consecutive nucleotides at or near the cleavage site of the strand. When the sense strand and the antisense strand form a dsRNA duplex, the sense strand and the antisense strand can be so aligned that one motif of the three nucleotides on the sense strand and one motif of the three nucleotides on the antisense strand have at least one nucleotide overlap, i.e., at least one of the three nucleotides of the motif in the sense strand forms a base pair with at least one of the three nucleotides of the motif in the antisense strand. Alternatively, at least two nucleotides may overlap, or all three nucleotides may overlap.

In some embodiments, the sense strand of the dsRNAi agent may contain more than one motif of three identical modifications on three consecutive nucleotides. The first motif may occur at or near the cleavage site of the strand and the other motifs may be a wing modification. The term "wing modification" herein refers to a motif occurring at another portion of the strand that is separated from the motif at or near the cleavage site of the same strand. The wing modification is either adjacent to the first motif or is separated by at least one or more nucleotides. When the motifs are immediately adjacent to each other then the chemistries of the motifs are distinct from each other, and when the motifs are separated by one or more nucleotide than the chemistries can be the same or different. Two or more wing modifications may be present. For instance, when two wing modifications are present, each wing modification may occur at one end relative to the first motif which is at or near cleavage site or on either side of the lead motif.

Like the sense strand, the antisense strand of the dsRNAi agent may contain more than one motif of three identical modifications on three consecutive nucleotides, with at least one of the motifs occurring at or near the cleavage site of the strand. This antisense strand may also contain one or more wing modifications in an alignment similar to the wing modifications that may be present on the sense strand.

In some embodiments, the wing modification on the sense strand or antisense strand of the dsRNAi agent typically does not include the first one or two terminal nucleotides at the 3'-end, 5'-end, or both ends of the strand.

In other embodiments, the wing modification on the sense strand or antisense strand of the dsRNAi agent typically does not include the first one or two paired nucleotides within the duplex region at the 3'-end, 5'-end, or both ends of the strand.

When the sense strand and the antisense strand of the dsRNAi agent each contain at least one wing modification, the wing modifications may fall on the same end of the duplex region, and have an overlap of one, two, or three nucleotides.

When the sense strand and the antisense strand of the dsRNAi agent each contain at least two wing modifications, the sense strand and the antisense strand can be so aligned that two modifications each from one strand fall on one end of the duplex region, having an overlap of one, two, or three nucleotides; two modifications each from one strand fall on the other end of the duplex region, having an overlap of one, two or three nucleotides; two modifications one strand fall on each side of the lead motif, having an overlap of one, two, or three nucleotides in the duplex region.

In some embodiments, every nucleotide in the sense strand and antisense strand of the dsRNAi agent, including the nucleotides that are part of the motifs, may be modified. Each nucleotide may be modified with the same or different modification which can include one or more alteration of one or both of the non-linking phosphate oxygens or of one or more of the linking phosphate oxygens; alteration of a constituent of the ribose sugar, e.g., of the 2'-hydroxyl on the ribose sugar; wholesale replacement of the phosphate moiety with "dephospho" linkers; modification or replacement of a naturally occurring base; and replacement or modification of the ribose-phosphate backbone.

As nucleic acids are polymers of subunits, many of the modifications occur at a position which is repeated within a nucleic acid, e.g., a modification of a base, or a phosphate

moiety, or a non-linking O of a phosphate moiety. In some cases the modification will occur at all of the subject positions in the nucleic acid but in many cases it will not. By way of example, a modification may only occur at a 3'- or 5'-terminal position, may only occur in a terminal region, e.g., at a position on a terminal nucleotide or in the last 2, 3, 4, 5, or 10 nucleotides of a strand. A modification may occur in a double strand region, a single strand region, or in both. A modification may occur only in the double strand region of a dsRNAi agent or may only occur in a single strand region of a dsRNAi agent. For example, a phosphorothioate modification at a non-linking O position may only occur at one or both ends, may only occur in a terminal region, e.g., at a position on a terminal nucleotide, or in the last 2, 3, 4, 5, or 10 nucleotides of a strand, or may occur in double strand and single strand regions, particularly at the ends. The 5'-end or ends can be phosphorylated.

It may be possible, e.g., to enhance stability, to include particular bases in overhangs, or to include modified nucleotides or nucleotide surrogates, in single strand overhangs, e.g., in a 5'- or 3'-overhang, or in both. For example, it can be desirable to include purine nucleotides in overhangs. In some embodiments all or some of the bases in a 3'- or 5'-overhang may be modified, e.g., with a modification described herein. Modifications can include, e.g., the use of modifications at the 2' position of the ribose sugar with modifications that are known in the art, e.g., the use of deoxyribonucleotides, 2'-deoxy-2'-fluoro (2'-F) or 2'-O-methyl modified instead of the ribosugar of the nucleobase, and modifications in the phosphate group, e.g., phosphorothioate modifications. Overhangs need not be homologous with the target sequence.

In some embodiments, each residue of the sense strand and antisense strand is independently modified with LNA, CRN, cET, UNA, HNA, CeNA, 2'-methoxyethyl, 2'-O-methyl, 2'-O-allyl, 2'-C-allyl, 2'-deoxy, 2'-hydroxyl, or 2'-fluoro. The strands can contain more than one modification. In one embodiment, each residue of the sense strand and antisense strand is independently modified with 2'-O-methyl or 2'-fluoro.

At least two different modifications are typically present on the sense strand and antisense strand. Those two modifications may be the 2'-O-methyl or 2'-fluoro modifications, or others. In certain embodiments, the N_a or N_b comprise modifications of an alternating pattern. The term "alternating motif" as used herein refers to a motif having one or more modifications, each modification occurring on alternating nucleotides of one strand. The alternating nucleotide may refer to one per every other nucleotide or one per every three nucleotides, or a similar pattern. For example, if A, B and C each represent one type of modification to the nucleotide, the alternating motif can be "ABABABABABAB . . . ," "AABBAABBAABB . . . ," "AABAABAABAAB . . . ," "AAABAAABAAAB . . . ," "AAABBBAAABBB . . . ," or "ABCABCABCABC . . . ," etc.

The type of modifications contained in the alternating motif may be the same or different. For example, if A, B, C, D each represent one type of modification on the nucleotide, the alternating pattern, i.e., modifications on every other nucleotide, may be the same, but each of the sense strand or antisense strand can be selected from several possibilities of modifications within the alternating motif such as "ABABAB . . . ", "ACACAC . . . " "BDBDBD . . . " or "CDCDCD . . . ," etc.

In some embodiments, the dsRNAi agent of the invention comprises the modification pattern for the alternating motif on the sense strand relative to the modification pattern for the alternating motif on the antisense strand is shifted. The shift may be such that the modified group of nucleotides of the sense strand corresponds to a differently modified group of nucleotides of the antisense strand and vice versa. For example, the sense strand when paired with the antisense strand in the dsRNA duplex, the alternating motif in the sense strand may start with "ABABAB" from 5' to 3' of the strand and the alternating motif in the antisense strand may start with "BABABA" from 5' to 3' of the strand within the duplex region. As another example, the alternating motif in the sense strand may start with "AABBAABB" from 5' to 3' of the strand and the alternating motif in the antisenese strand may start with "BBAABBAA" from 5' to 3' of the strand within the duplex region, so that there is a complete or partial shift of the modification patterns between the sense strand and the antisense strand.

In some embodiments, the dsRNAi agent comprises the pattern of the alternating motif of 2'-O-methyl modification and 2'-F modification on the sense strand initially has a shift relative to the pattern of the alternating motif of 2'-O-methyl modification and 2'-F modification on the antisense strand initially, i.e., the 2'-O-methyl modified nucleotide on the sense strand base pairs with a 2'-F modified nucleotide on the antisense strand and vice versa. The 1 position of the sense strand may start with the 2'-F modification, and the 1 position of the antisense strand may start with the 2'-O-methyl modification.

The introduction of one or more motifs of three identical modifications on three consecutive nucleotides to the sense strand or antisense strand interrupts the initial modification pattern present in the sense strand or antisense strand. This interruption of the modification pattern of the sense or antisense strand by introducing one or more motifs of three identical modifications on three consecutive nucleotides to the sense or antisense strand may enhance the gene silencing activity against the target gene.

In some embodiments, when the motif of three identical modifications on three consecutive nucleotides is introduced to any of the strands, the modification of the nucleotide next to the motif is a different modification than the modification of the motif. For example, the portion of the sequence containing the motif is " . . . N_aYYYN_b . . . ," where "Y" represents the modification of the motif of three identical modifications on three consecutive nucleotide, and "N_a" and "N_b" represent a modification to the nucleotide next to the motif "YYY" that is different than the modification of Y, and where N_a and N_b can be the same or different modifications. Alternatively, N_a or N_b may be present or absent when there is a wing modification present.

The iRNA may further comprise at least one phosphorothioate or methylphosphonate internucleotide linkage. The phosphorothioate or methylphosphonate internucleotide linkage modification may occur on any nucleotide of the sense strand, antisense strand, or both strands in any position of the strand. For instance, the internucleotide linkage modification may occur on every nucleotide on the sense strand or antisense strand; each internucleotide linkage modification may occur in an alternating pattern on the sense strand or antisense strand; or the sense strand or antisense strand may contain both internucleotide linkage modifications in an alternating pattern. The alternating pattern of the internucleotide linkage modification on the sense strand may be the same or different from the antisense strand, and the alternating pattern of the internucleotide linkage modification on the sense strand may have a shift relative to the alternating pattern of the internucleotide linkage modification on the antisense strand. In one embodiment, a double-

stranded RNAi agent comprises 6-8phosphorothioate internucleotide linkages. In some embodiments, the antisense strand comprises two phosphorothioate internucleotide linkages at the 5'-end and two phosphorothioate internucleotide linkages at the 3'-end, and the sense strand comprises at least two phosphorothioate internucleotide linkages at either the 5'-end or the 3'-end.

In some embodiments, the dsRNAi agent comprises a phosphorothioate or methylphosphonate internucleotide linkage modification in the overhang region. For example, the overhang region may contain two nucleotides having a phosphorothioate or methylphosphonate internucleotide linkage between the two nucleotides. Internucleotide linkage modifications also may be made to link the overhang nucleotides with the terminal paired nucleotides within the duplex region. For example, at least 2, 3, 4, or all the overhang nucleotides may be linked through phosphorothioate or methylphosphonate internucleotide linkage, and optionally, there may be additional phosphorothioate or methylphosphonate internucleotide linkages linking the overhang nucleotide with a paired nucleotide that is next to the overhang nucleotide. For instance, there may be at least two phosphorothioate internucleotide linkages between the terminal three nucleotides, in which two of the three nucleotides are overhang nucleotides, and the third is a paired nucleotide next to the overhang nucleotide. These terminal three nucleotides may be at the 3'-end of the antisense strand, the 3'-end of the sense strand, the 5'-end of the antisense strand, or the 5' end of the antisense strand.

In some embodiments, the 2-nucleotide overhang is at the 3'-end of the antisense strand, and there are two phosphorothioate internucleotide linkages between the terminal three nucleotides, wherein two of the three nucleotides are the overhang nucleotides, and the third nucleotide is a paired nucleotide next to the overhang nucleotide. Optionally, the dsRNAi agent may additionally have two phosphorothioate internucleotide linkages between the terminal three nucleotides at both the 5'-end of the sense strand and at the 5'-end of the antisense strand.

In one embodiment, the dsRNAi agent comprises mismatch(es) with the target, within the duplex, or combinations thereof. The mismatch may occur in the overhang region or the duplex region. The base pair may be ranked on the basis of their propensity to promote dissociation or melting (e.g., on the free energy of association or dissociation of a particular pairing, the simplest approach is to examine the pairs on an individual pair basis, though next neighbor or similar analysis can also be used). In terms of promoting dissociation: A:U is preferred over G:C; G:U is preferred over G:C; and I:C is preferred over G:C (I=inosine). Mismatches, e.g., non-canonical or other than canonical pairings (as described elsewhere herein) are preferred over canonical (A:T, A:U, G:C) pairings; and pairings which include a universal base are preferred over canonical pairings.

In certain embodiments, the dsRNAi agent comprises at least one of the first 1, 2, 3, 4, or 5 base pairs within the duplex regions from the 5'-end of the antisense strand independently selected from the group of: A:U, G:U, I:C, and mismatched pairs, e.g., non-canonical or other than canonical pairings or pairings which include a universal base, to promote the dissociation of the antisense strand at the 5'-end of the duplex.

In certain embodiments, the nucleotide at the 1 position within the duplex region from the 5'-end in the antisense strand is selected from A, dA, dU, U, and dT. Alternatively, at least one of the first 1, 2, or 3 base pair within the duplex region from the 5'-end of the antisense strand is an AU base pair. For example, the first base pair within the duplex region from the 5'-end of the antisense strand is an AU base pair.

In other embodiments, the nucleotide at the 3'-end of the sense strand is deoxy-thymine (dT) or the nucleotide at the 3'-end of the antisense strand is deoxy-thymine (dT). For example, there is a short sequence of deoxy-thymine nucleotides, for example, two dT nucleotides on the 3'-end of the sense, antisense strand, or both strands.

In certain embodiments, the sense strand sequence may be represented by formula (I):

$$5'n_p\text{—}N_a\text{—}(XXX)_i\text{—}N_b\text{—}YYY\text{—}N_b\text{—}(ZZZ)_i\text{—}N_a\text{-}n_q3' \quad (I)$$

wherein:

i and j are each independently 0 or 1;

p and q are each independently 0-6;

each N_a independently represents an oligonucleotide sequence comprising 0-25 modified nucleotides, each sequence comprising at least two differently modified nucleotides;

each N_b independently represents an oligonucleotide sequence comprising 0-10 modified nucleotides;

each n_p and n_q independently represent an overhang nucleotide;

wherein Nb and Y do not have the same modification; and

XXX, YYY, and ZZZ each independently represent one motif of three identical modifications on three consecutive nucleotides. Preferably YYY is all 2'-F modified nucleotides.

In some embodiments, the N_a or N_b comprises modifications of alternating pattern.

In some embodiments, the YYY motif occurs at or near the cleavage site of the sense strand. For example, when the dsRNAi agent has a duplex region of 17-23 nucleotides in length, the YYY motif can occur at or the vicinity of the cleavage site (e.g.: can occur at positions 6, 7, 8; 7, 8, 9; 8, 9, 10; 9, 10, 11; 10, 11,12; or 11, 12, 13) of the sense strand, the count starting from the first nucleotide, from the 5'-end; or optionally, the count starting at the first paired nucleotide within the duplex region, from the 5'-end.

In one embodiment, i is 1 and j is 0, or i is 0 and j is 1, or both i and j are 1. The sense strand can therefore be represented by the following formulas:

$$5'n_p\text{—}N_a\text{—}YYY\text{—}N_b\text{—}ZZZ\text{—}N_a\text{-}n_q3' \quad (Ib);$$

$$5'n_p\text{—}N_a\text{—}XXX\text{—}N_b\text{—}YYY\text{—}N_a\text{-}n_q3' \quad (Ic); \text{ or}$$

$$5'n_p\text{—}N_a\text{—}XXX\text{—}N_b\text{—}YYY\text{—}N_b\text{—}ZZZ\text{—}N_a\text{-}n_q3' \quad (Id).$$

When the sense strand is represented by formula (Ib), N_b represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleotides. Each N_a independently can represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the sense strand is represented as formula (Ic), N_b represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleotides. Each N_a can independently represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the sense strand is represented as formula (Id), each N_b independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleotides. Preferably, N_b is 0, 1, 2, 3, 4, 5, or 6 Each N_a can independently represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

Each of X, Y and Z may be the same or different from each other.

In other embodiments, i is 0 and j is 0, and the sense strand may be represented by the formula:

$$5'n_p—N_a—YYY—N_a\text{-}n_q3' \quad \text{(Ia).}$$

When the sense strand is represented by formula (Ia), each N_a independently can represent an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

In one embodiment, the antisense strand sequence of the RNAi may be represented by formula (II):

$$5'n_{q'}—N_a'—(Z'Z'Z')_k—N_b'—Y'Y'—N_b'—(X'X'X')_l—N'_a\text{-}n_p'3' \quad \text{(II)}$$

wherein:

k and l are each independently 0 or 1;

p' and q' are each independently 0-6;

each N_a' independently represents an oligonucleotide sequence comprising 0-25 modified nucleotides, each sequence comprising at least two differently modified nucleotides;

each N_b' independently represents an oligonucleotide sequence comprising 0-10 modified nucleotides;

each n_p' and n_q' independently represent an overhang nucleotide;

wherein N_b' and Y' do not have the same modification; and

X'X'X', Y'Y'Y', and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides.

In some embodiments, the N_a' or N_b' comprises modifications of alternating pattern.

The Y'Y'Y' motif occurs at or near the cleavage site of the antisense strand. For example, when the dsRNAi agent has a duplex region of 17-23 nucleotides in length, the Y'Y'Y' motif can occur at positions 9, 10, 11; 10, 11, 12; 11, 12, 13; 12, 13, 14; or 13, 14, 15 of the antisense strand, with the count starting from the first nucleotide, from the 5'-end; or optionally, the count starting at the first paired nucleotide within the duplex region, from the 5'-end. Preferably, the Y'Y'Y' motif occurs at positions 11, 12, 13.

In certain embodiments, Y'Y'Y' motif is all 2'-OMe modified nucleotides.

In certain embodiments, k is 1 and 1 is 0, or k is 0 and 1 is 1, or both k and 1 are 1.

The antisense strand can therefore be represented by the following formulas:

$$5'n_{q'}—N_a'—Z'Z'Z'—N_b'—Y'Y'Y'—N_{a'}\text{-}n_p'3' \quad \text{(IIb);}$$

$$5'n_{q'}—N_a'—Y'Y'Y'—N_b'—X'X'X'\text{-}n_p'3' \quad \text{(IIc); or}$$

$$5'n_{q'}—N_a'—Z'Z'Z'—N_b'—Y'Y'Y'—N_b'—X'X'X'—N_a'\text{-}n_p'3' \quad \text{(IId).}$$

When the antisense strand is represented by formula (IIb), N_b' represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleotides. Each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the antisense strand is represented as formula (IIc), N_b' represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleotides. Each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the antisense strand is represented as formula (IId), each N_b' independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleotides. Each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides. Preferably, N_b is 0, 1, 2, 3, 4, 5, or 6.

In other embodiments, k is 0 and 1 is 0 and the antisense strand may be represented by the formula:

$$5'n_{p'}—N_a—Y'Y'Y'—N_a\text{-}n_{q'}3' \quad \text{(Ia).}$$

When the antisense strand is represented as formula (IIa), each N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides. Each of X', Y' and Z' may be the same or different from each other.

Each nucleotide of the sense strand and antisense strand may be independently modified with LNA, CRN, UNA, cEt, HNA, CeNA, 2'-methoxyethyl, 2'-O-methyl, 2'-O-allyl, 2'-C-allyl, 2'-hydroxyl, or 2'-fluoro. For example, each nucleotide of the sense strand and antisense strand is independently modified with 2'-O-methyl or 2'-fluoro. Each X, Y, Z, X', Y', and Z', in particular, may represent a 2'-O-methyl modification or a 2'-fluoro modification.

In some embodiments, the sense strand of the dsRNAi agent may contain YYY motif occurring at 9, 10, and 11 positions of the strand when the duplex region is 21 nt, the count starting from the first nucleotide from the 5'-end, or optionally, the count starting at the first paired nucleotide within the duplex region, from the 5'-end; and Y represents 2'-F modification. The sense strand may additionally contain XXX motif or ZZZ motifs as wing modifications at the opposite end of the duplex region; and XXX and ZZZ each independently represents a 2'-OMe modification or 2'-F modification.

In some embodiments the antisense strand may contain Y'Y'Y' motif occurring at positions 11, 12, 13 of the strand, the count starting from the first nucleotide from the 5'-end, or optionally, the count starting at the first paired nucleotide within the duplex region, from the 5'-end; and Y' represents 2'-O-methyl modification. The antisense strand may additionally contain X'X'X' motif or Z'Z'Z' motifs as wing modifications at the opposite end of the duplex region; and X'X'X' and Z'Z'Z' each independently represents a 2'—OMe modification or 2'—F modification.

The sense strand represented by any one of the above formulas (Ia), (Ib), (Ic), and (Id) forms a duplex with a antisense strand being represented by any one of formulas (IIa), (IIb), (IIc), and (IId), respectively.

Accordingly, the dsRNAi agents for use in the methods of the invention may comprise a sense strand and an antisense strand, each strand having 14 to 30 nucleotides, the iRNA duplex represented by formula (III):

$$\text{sense: } 5'n_p—N_a—(XXX)_i—N_b—YYY—N_b—(ZZZ)_j—N_a\text{-}n_q3'$$

$$\text{antisense: } 3'n_p'—N_a'—(X'X'X')_k—N_b'—Y'Y'Y'—N_b'—(Z'Z'Z')_l—N_{a'}\text{-}n_q'5' \quad \text{(III)}$$

wherein:

j, k, and l are each independently 0 or 1;

p, p', q, and q' are each independently 0-6;

each N_a and N_a' independently represents an oligonucleotide sequence comprising 0-25 modified nucleotides, each sequence comprising at least two differently modified nucleotides;

each N_b and N_b' independently represents an oligonucleotide sequence comprising 0-10 modified nucleotides;

wherein each n_p', n_p, n_q', and n_q, each of which may or may not be present, independently represents an overhang nucleotide; and

XXX, YYY, ZZZ, X'X'X', Y'Y'Y', and Z'Z'Z' each independently represent one motif of three identical modifications on three consecutive nucleotides.

In one embodiment, i is 0 and j is 0; or i is 1 and j is 0; or i is 0 and j is 1; or both i and j are 0; or both i and j are 1. In another embodiment, k is 0 and 1 is 0; or k is 1 and 1 is 0; k is 0 and 1 is 1; or both k and 1 are 0; or both k and 1 are 1.

Exemplary combinations of the sense strand and antisense strand forming an iRNA duplex include the formulas below:

$5'n_p$—N_a—YYY—N_a-n_q3'

$3'n_p$'—N_a'—Y'Y'Y'—$N_a n_q$'5' (IIIa)

$5'n_p$—N_a—YYY—N_b—ZZZ—N_a-n_q3'

$3'n_p$'—N_a'—Y'Y'Y'—N_b'—Z'Z'Z'—$N_a n_q$'5' (IIIb)

$5'n_p$—N_a—XXX—N_b—YYY—N_a-n_q3'

$3'n_p$'—N_a'—X'X'X'—N_b'—Y'Y'Y'—$N_{a'}$-n_q'5' (IIIc)

$5'n_p$—N_a—XXX—N_b—YYY—N_b—ZZZ—N_a-n_q3'

$3'n_p$'—N_a'—X'X'X'—N_b'—Y'Y'Y'—N_b'—Z'Z'Z'—
N_a-n_q'5' (IIId)

When the dsRNAi agent is represented by formula (IIIa), each N_a independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the dsRNAi agent is represented by formula (IIIb), each N_b independently represents an oligonucleotide sequence comprising 1-10, 1-7, 1-5, or 1-4 modified nucleotides. Each N_a independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the dsRNAi agent is represented as formula (IIIc), each N_b, N_b' independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleotides. Each N_a independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides.

When the dsRNAi agent is represented as formula (IIId), each N_b, N_b' independently represents an oligonucleotide sequence comprising 0-10, 0-7, 0-10, 0-7, 0-5, 0-4, 0-2, or 0 modified nucleotides. Each N_a, N_a' independently represents an oligonucleotide sequence comprising 2-20, 2-15, or 2-10 modified nucleotides. Each of N_a, N_a', N_b, and N_b' independently comprises modifications of alternating pattern.

Each of X, Y, and Z in formulas (III), (IIIa), (IIIb), (IIIc), and (IIId) may be the same or different from each other.

When the dsRNAi agent is represented by formula (III), (IIIa), (IIIb), (IIIc), and (IIId), at least one of the Y nucleotides may form a base pair with one of the Y' nucleotides. Alternatively, at least two of the Y nucleotides form base pairs with the corresponding Y' nucleotides; or all three of the Y nucleotides all form base pairs with the corresponding Y' nucleotides.

When the dsRNAi agent is represented by formula (IIIb) or (IIId), at least one of the Z nucleotides may form a base pair with one of the Z' nucleotides. Alternatively, at least two of the Z nucleotides form base pairs with the corresponding Z' nucleotides; or all three of the Z nucleotides all form base pairs with the corresponding Z' nucleotides.

When the dsRNAi agent is represented as formula (IIIc) or (IIId), at least one of the X nucleotides may form a base pair with one of the X' nucleotides. Alternatively, at least two of the X nucleotides form base pairs with the corresponding X' nucleotides; or all three of the X nucleotides all form base pairs with the corresponding X' nucleotides.

In certain embodiments, the modification on the Y nucleotide is different than the modification on the Y' nucleotide, the modification on the Z nucleotide is different than the modification on the Z' nucleotide, or the modification on the X nucleotide is different than the modification on the X' nucleotide.

In certain embodiments, when the dsRNAi agent is represented by formula (IIId), the N_a modifications are 2'-O-methyl or 2'-fluoro modifications. In other embodiments, when the RNAi agent is represented by formula (IIId), the N_a modifications are 2'-O-methyl or 2'-fluoro modifications and n_p'>0 and at least one n_p' is linked to a neighboring nucleotide a via phosphorothioate linkage. In yet other embodiments, when the RNAi agent is represented by formula (IIId), the N_a modifications are 2'-O-methyl or 2'-fluoro modifications, n_p'>0 and at least one n_p' is linked to a neighboring nucleotide via phosphorothioate linkage, and the sense strand is conjugated to one or more GalNAc derivatives attached through a bivalent or trivalent branched linker (described below). In other embodiments, when the RNAi agent is represented by formula (IIId), the N_a modifications are 2'-O-methyl or 2'-fluoro modifications, n_p'>0 and at least one n_p' is linked to a neighboring nucleotide via phosphorothioate linkage, the sense strand comprises at least one phosphorothioate linkage, and the sense strand is conjugated to one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

In some embodiments, when the dsRNAi agent is represented by formula (IIIa), the N_a modifications are 2'-O-methyl or 2'-fluoro modifications, n_p'>0 and at least one n_p' is linked to a neighboring nucleotide via phosphorothioate linkage, the sense strand comprises at least one phosphorothioate linkage, and the sense strand is conjugated to one or more GalNAc derivatives attached through a bivalent or trivalent branched linker.

In some embodiments, the dsRNAi agent is a multimer containing at least two duplexes represented by formula (III), (IIIa), (IIIb), (IIIc), and (IIId), wherein the duplexes are connected by a linker The linker can be cleavable or non-cleavable. Optionally, the multimer further comprises a ligand. Each of the duplexes can target the same gene or two different genes; or each of the duplexes can target same gene at two different target sites.

In some embodiments, the dsRNAi agent is a multimer containing three, four, five, six, or more duplexes represented by formula (III), (IIIa), (IIIb), (IIIc), and (IIId), wherein the duplexes are connected by a linker The linker can be cleavable or non-cleavable. Optionally, the multimer further comprises a ligand. Each of the duplexes can target the same gene or two different genes; or each of the duplexes can target same gene at two different target sites.

In one embodiment, two dsRNAi agents represented by at least one of formulas (III), (IIIa), (IIIb), (IIIc), and (IIId) are linked to each other at the 5' end, and one or both of the 3' ends, and are optionally conjugated to to a ligand. Each of the agents can target the same gene or two different genes; or each of the agents can target same gene at two different target sites.

Various publications describe multimeric iRNAs that can be used in the methods of the invention. Such publications include WO2007/091269, U.S. Pat. No. 7,858,769, WO2010/141511, WO2007/117686, WO2009/014887, and WO2011/031520 the entire contents of each of which are hereby incorporated herein by reference.

As described in more detail below, the iRNA that contains conjugations of one or more carbohydrate moieties to an iRNA can optimize one or more properties of the iRNA. In many cases, the carbohydrate moiety will be attached to a modified subunit of the iRNA. For example, the ribose sugar of one or more ribonucleotide subunits of a iRNA can be replaced with another moiety, e.g., a non-carbohydrate (preferably cyclic) carrier to which is attached a carbohydrate ligand. A ribonucleotide subunit in which the ribose sugar of the subunit has been so replaced is referred to herein as a ribose replacement modification subunit (RRMS). A cyclic carrier may be a carbocyclic ring system, i.e., all ring atoms are carbon atoms, or a heterocyclic ring system, i.e., one or more ring atoms may be a heteroatom, e.g., nitrogen, oxygen, sulfur. The cyclic carrier may be a monocyclic ring system, or may contain two or more rings, e.g. fused rings. The cyclic carrier may be a fully saturated ring system, or it may contain one or more double bonds.

The ligand may be attached to the polynucleotide via a carrier. The carriers include (i) at least one "backbone attachment point," preferably two "backbone attachment points" and (ii) at least one "tethering attachment point." A "backbone attachment point" as used herein refers to a functional group, e.g. a hydroxyl group, or generally, a bond available for, and that is suitable for incorporation of the carrier into the backbone, e.g., the phosphate, or modified phosphate, e.g., sulfur containing, backbone, of a ribonucleic acid. A "tethering attachment point" (TAP) in some embodiments refers to a constituent ring atom of the cyclic carrier, e.g., a carbon atom or a heteroatom (distinct from an atom which provides a backbone attachment point), that connects a selected moiety. The moiety can be, e.g., a carbohydrate, e.g. monosaccharide, disaccharide, trisaccharide, tetrasaccharide, oligosaccharide, or polysaccharide. Optionally, the selected moiety is connected by an intervening tether to the cyclic carrier. Thus, the cyclic carrier will often include a functional group, e.g., an amino group, or generally, provide a bond, that is suitable for incorporation or tethering of another chemical entity, e.g., a ligand to the constituent ring.

The iRNA may be conjugated to a ligand via a carrier, wherein the carrier can be cyclic group or acyclic group; preferably, the cyclic group is selected from pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, [1,3]dioxolane, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, quinoxalinyl, pyridazinonyl, tetrahydrofuryl, and decalin; preferably, the acyclic group is a serinol backbone or diethanolamine backbone.

In certain embodiments, the iRNA for use in the methods of the invention is an agent selected from agents listed in Table 3, Table 4, Table 6 or Table 7. These agents may further comprise a ligand.

III. iRNAs Conjugated to Ligands

Another modification of the RNA of an iRNA of the invention involves chemically linking to the iRNA one or more ligands, moieties or conjugates that enhance the activity, cellular distribution, or cellular uptake of the iRNA e.g., into a cell. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger et al., *Proc. Natl. Acid. Sci. USA,* 1989, 86: 6553-6556), cholic acid (Manoharan et al., *Biorg. Med. Chem. Let.,* 1994, 4:1053-1060), a thioether, e.g., beryl-S-tritylthiol (Manoharan et al., *Ann. N.Y. Acad. Sci.,* 1992, 660:306-309; Manoharan et al., *Biorg. Med. Chem. Let.,* 1993, 3:2765-2770), a thiocholesterol (Oberhauser et al., *Nucl. Acids Res.,* 1992, 20:533-538), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., *EMBO J,* 1991, 10:1111-1118; Kabanov et al., *FEBS Lett.,* 1990, 259:327-330; Svinarchuk et al., *Biochimie,* 1993, 75:49-54), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-phosphonate (Manoharan et al., *Tetrahedron Lett.,* 1995, 36:3651-3654; Shea et al., *Nucl. Acids Res.,* 1990, 18:3777-3783), a polyamine or a polyethylene glycol chain (Manoharan et al., *Nucleosides & Nucleotides,* 1995, 14:969-973), or adamantane acetic acid (Manoharan et al., *Tetrahedron Lett.,* 1995, 36:3651-3654), a palmityl moiety (Mishra et al., *Biochim. Biophys. Acta,* 1995, 1264:229-237), or an octadecylamine or hexylamino-carbonyloxycholesterol moiety (Crooke et al., *J. Pharmacol. Exp. Ther.,* 1996, 277:923-937).

In certain embodiments, a ligand alters the distribution, targeting or lifetime of an iRNA agent into which it is incorporated. In preferred embodiments a ligand provides an enhanced affinity for a selected target, e.g., molecule, cell or cell type, compartment, e.g., a cellular or organ compartment, tissue, organ or region of the body, as, e.g., compared to a species absent such a ligand. Preferred ligands do not take part in duplex pairing in a duplexed nucleic acid.

Ligands can include a naturally occurring substance, such as a protein (e.g., human serum albumin (HSA), low-density lipoprotein (LDL), or globulin); carbohydrate (e.g., a dextran, pullulan, chitin, chitosan, inulin, cyclodextrin, N-acetylgalactosamine, or hyaluronic acid); or a lipid. The ligand can also be a recombinant or synthetic molecule, such as a synthetic polymer, e.g., a synthetic polyamino acid. Examples of polyamino acids include polyamino acid is a polylysine (PLL), poly L-aspartic acid, poly L-glutamic acid, styrene-maleic acid anhydride copolymer, poly(L-lactide-co-glycolied) copolymer, divinyl ether-maleic anhydride copolymer, N-(2-hydroxypropyl)methacrylamide copolymer (HMPA), polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyurethane, poly(2-ethylacryllic acid), N-isopropylacrylamide polymers, or polyphosphazine Example of polyamines include: polyethylenimine, polylysine (PLL), spermine, spermidine, polyamine, pseudopeptide-polyamine, peptidomimetic polyamine, dendrimer polyamine, arginine, amidine, protamine, cationic lipid, cationic porphyrin, quaternary salt of a polyamine, or an alpha helical peptide.

Ligands can also include targeting groups, e.g., a cell or tissue targeting agent, e.g., a lectin, glycoprotein, lipid or protein, e.g., an antibody, that binds to a specified cell type such as a kidney cell. A targeting group can be a thyrotropin, melanotropin, lectin, glycoprotein, surfactant protein A, Mucin carbohydrate, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-glucoseamine multivalent mannose, multivalent fucose, glycosylated polyaminoacids, galactose, transferrin, bisphosphonate, polyglutamate, polyaspartate, a lipid, cholesterol, a steroid, bile acid, folate, vitamin B12, vitamin A, biotin, or an RGD peptide or RGD peptide mimetic.

Other examples of ligands include dyes, intercalating agents (e.g. acridines), cross-linkers (e.g. psoralen, mitomycin C), porphyrins (TPPC4, texaphyrin, Sapphyrin), polycyclic aromatic hydrocarbons (e.g., phenazine, dihydrophenazine), artificial endonucleases (e.g. EDTA), lipophilic molecules, e.g., cholesterol, cholic acid, adamantane acetic acid, 1-pyrene butyric acid, dihydrotestosterone, 1,3-Bis-O (hexadecyl)glycerol, geranyloxyhexyl group, hexadecylglycerol, borneol, menthol, 1,3-propanediol, heptadecyl group, palmitic acid, myristic acid,O3-(oleoyl)

lithocholic acid, O3-(oleoyl)cholenic acid, dimethoxytrityl, or phenoxazine) and peptide conjugates (e.g., antennapedia peptide, Tat peptide), alkylating agents, phosphate, amino, mercapto, PEG (e.g., PEG-40K), MPEG, $[MPEG]_2$, polyamino, alkyl, substituted alkyl, radiolabeled markers, enzymes, haptens (e.g. biotin), transport/absorption facilitators (e.g., aspirin, vitamin E, folic acid), synthetic ribonucleases (e.g., imidazole, bisimidazole, histamine, imidazole clusters, acridine-imidazole conjugates, Eu3+ complexes of tetraazamacrocycles), dinitrophenyl, HRP, or AP.

Ligands can be proteins, e.g., glycoproteins, or peptides, e.g., molecules having a specific affinity for a co-ligand, or antibodies e.g., an antibody, that binds to a specified cell type such as a hepatic cell. Ligands can also include hormones and hormone receptors. They can also include non-peptidic species, such as lipids, lectins, carbohydrates, vitamins, cofactors, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-gulucosamine multivalent mannose, or multivalent fucose. The ligand can be, for example, a lipopolysaccharide, an activator of p38 MAP kinase, or an activator of NF-κB.

The ligand can be a substance, e.g., a drug, which can increase the uptake of the iRNA agent into the cell, for example, by disrupting the cell's cytoskeleton, e.g., by disrupting the cell's microtubules, microfilaments, or intermediate filaments. The drug can be, for example, taxon, vincristine, vinblastine, cytochalasin, nocodazole, japlakinolide, latrunculin A, phalloidin, swinholide A, indanocine, or myoservin.

In some embodiments, a ligand attached to an iRNA as described herein acts as a pharmacokinetic modulator (PK modulator). PK modulators include lipophiles, bile acids, steroids, phospholipid analogues, peptides, protein binding agents, PEG, vitamins etc. Exemplary PK modulators include, but are not limited to, cholesterol, fatty acids, cholic acid, lithocholic acid, dialkylglycerides, diacylglyceride, phospholipids, sphingolipids, naproxen, ibuprofen, vitamin E, biotin etc. Oligonucleotides that comprise a number of phosphorothioate linkages are also known to bind to serum protein, thus short oligonucleotides, e.g., oligonucleotides of about 5 bases, 10 bases, 15 bases, or 20 bases, comprising multiple of phosphorothioate linkages in the backbone are also amenable to the present invention as ligands (e.g. as PK modulating ligands). In addition, aptamers that bind serum components (e.g. serum proteins) are also suitable for use as PK modulating ligands in the embodiments described herein.

Ligand-conjugated iRNAs of the invention may be synthesized by the use of an oligonucleotide that bears a pendant reactive functionality, such as that derived from the attachment of a linking molecule onto the oligonucleotide (described below). This reactive oligonucleotide may be reacted directly with commercially-available ligands, ligands that are synthesized bearing any of a variety of protecting groups, or ligands that have a linking moiety attached thereto.

The oligonucleotides used in the conjugates of the present invention may be conveniently and routinely made through the well-known technique of solid-phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems (Foster City, Calif.). Any other means for such synthesis known in the art may additionally or alternatively be employed. It is also known to use similar techniques to prepare other oligonucleotides, such as the phosphorothioates and alkylated derivatives.

In the ligand-conjugated iRNAs and ligand-molecule bearing sequence-specific linked nucleosides of the present invention, the oligonucleotides and oligonucleosides may be assembled on a suitable DNA synthesizer utilizing standard nucleotide or nucleoside precursors, or nucleotide or nucleoside conjugate precursors that already bear the linking moiety, ligand-nucleotide or nucleoside-conjugate precursors that already bear the ligand molecule, or non-nucleoside ligand-bearing building blocks.

When using nucleotide-conjugate precursors that already bear a linking moiety, the synthesis of the sequence-specific linked nucleosides is typically completed, and the ligand molecule is then reacted with the linking moiety to form the ligand-conjugated oligonucleotide. In some embodiments, the oligonucleotides or linked nucleosides of the present invention are synthesized by an automated synthesizer using phosphoramidites derived from ligand-nucleoside conjugates in addition to the standard phosphoramidites and non-standard phosphoramidites that are commercially available and routinely used in oligonucleotide synthesis.

A. Lipid Conjugates

In certain embodiments, the ligand or conjugate is a lipid or lipid-based molecule. Such a lipid or lipid-based molecule preferably binds a serum protein, e.g., human serum albumin (HSA). An HSA binding ligand allows for distribution of the conjugate to a target tissue, e.g., a non-kidney target tissue of the body. For example, the target tissue can be the liver, including parenchymal cells of the liver. Other molecules that can bind HSA can also be used as ligands. For example, naproxen or aspirin can be used. A lipid or lipid-based ligand can (a) increase resistance to degradation of the conjugate, (b) increase targeting or transport into a target cell or cell membrane, or (c) can be used to adjust binding to a serum protein, e.g., HSA.

A lipid based ligand can be used to inhibit, e.g., control the binding of the conjugate to a target tissue. For example, a lipid or lipid-based ligand that binds to HSA more strongly will be less likely to be targeted to the kidney and therefore less likely to be cleared from the body. A lipid or lipid-based ligand that binds to HSA less strongly can be used to target the conjugate to the kidney.

In certain embodiments, the lipid based ligand binds HSA. Preferably, it binds HSA with a sufficient affinity such that the conjugate will be preferably distributed to a non-kidney tissue. However, it is preferred that the affinity not be so strong that the HSA-ligand binding cannot be reversed.

In other embodiments, the lipid based ligand binds HSA weakly or not at all, such that the conjugate will be preferably distributed to the kidney. Other moieties that target to kidney cells can also be used in place of, or in addition to, the lipid based ligand.

In another aspect, the ligand is a moiety, e.g., a vitamin, which is taken up by a target cell, e.g., a proliferating cell. These are particularly useful for treating disorders characterized by unwanted cell proliferation, e.g., of the malignant or non-malignant type, e.g., cancer cells. Exemplary vitamins include vitamin A, E, and K. Other exemplary vitamins include are B vitamin, e.g., folic acid, B12, riboflavin, biotin, pyridoxal or other vitamins or nutrients taken up by target cells such as liver cells. Also included are HSA and low density lipoprotein (LDL).

B. Cell Permeation Agents In another aspect, the ligand is a cell-permeation agent, preferably a helical cell-permeation agent. Preferably, the agent is amphipathic. An exemplary agent is a peptide such as tat or antennopedia. If the agent is a peptide, it can be modified, including a peptidylmimetic, invertomers, non-peptide or pseudo-peptide linkages, and use of D-amino acids. The helical agent is preferably an alpha-helical agent, which preferably has a lipophilic and a lipophobic phase.

The ligand can be a peptide or peptidomimetic. A peptidomimetic (also referred to herein as an oligopeptidomimetic) is a molecule capable of folding into a defined three-dimensional structure similar to a natural peptide. The

attachment of peptide and peptidomimetics to iRNA agents can affect pharmacokinetic distribution of the iRNA, such as by enhancing cellular recognition and absorption. The peptide or peptidomimetic moiety can be about 5-50 amino acids long, e.g., about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 amino acids long.

A peptide or peptidomimetic can be, for example, a cell permeation peptide, cationic peptide, amphipathic peptide, or hydrophobic peptide (e.g., consisting primarily of Tyr, Trp, or Phe). The peptide moiety can be a dendrimer peptide, constrained peptide or crosslinked peptide. In another alternative, the peptide moiety can include a hydrophobic membrane translocation sequence (MTS). An exemplary hydrophobic MTS-containing peptide is RFGF having the amino acid sequence AAVALLPAVLLALLAP (SEQ ID NO:11). An RFGF analogue (e.g., amino acid sequence AALLPVLLAAP (SEQ ID NO:12) containing a hydrophobic MTS can also be a targeting moiety. The peptide moiety can be a "delivery" peptide, which can carry large polar molecules including peptides, oligonucleotides, and protein across cell membranes. For example, sequences from the HIV Tat protein (GRKKRRQRRRPPQ (SEQ ID NO:13) and the *Drosophila* Antennapedia protein (RQIKIWFQNRRMKWKK (SEQ ID NO:14) have been found to be capable of functioning as delivery peptides. A peptide or peptidomimetic can be encoded by a random sequence of DNA, such as a peptide identified from a phage-display library, or one-bead-one-compound (OBOC) combinatorial library (Lam et al., Nature, 354:82-84, 1991). Examples of a peptide or peptidomimetic tethered to a dsRNA agent via an incorporated monomer unit for cell targeting purposes is an arginine-glycine-aspartic acid (RGD)-peptide, or RGD mimic. A peptide moiety can range in length from about 5 amino acids to about 40 amino acids. The peptide moieties can have a structural modification, such as to increase stability or direct conformational properties. Any of the structural modifications described below can be utilized

An RGD peptide for use in the compositions and methods of the invention may be linear or cyclic, and may be modified, e.g., glycosylated or methylated, to facilitate targeting to a specific tissue(s). RGD-containing peptides and peptidiomimemtics may include D-amino acids, as well as synthetic RGD mimics. In addition to RGD, one can use other moieties that target the integrin ligand. Preferred conjugates of this ligand target PECAM-1 or VEGF.

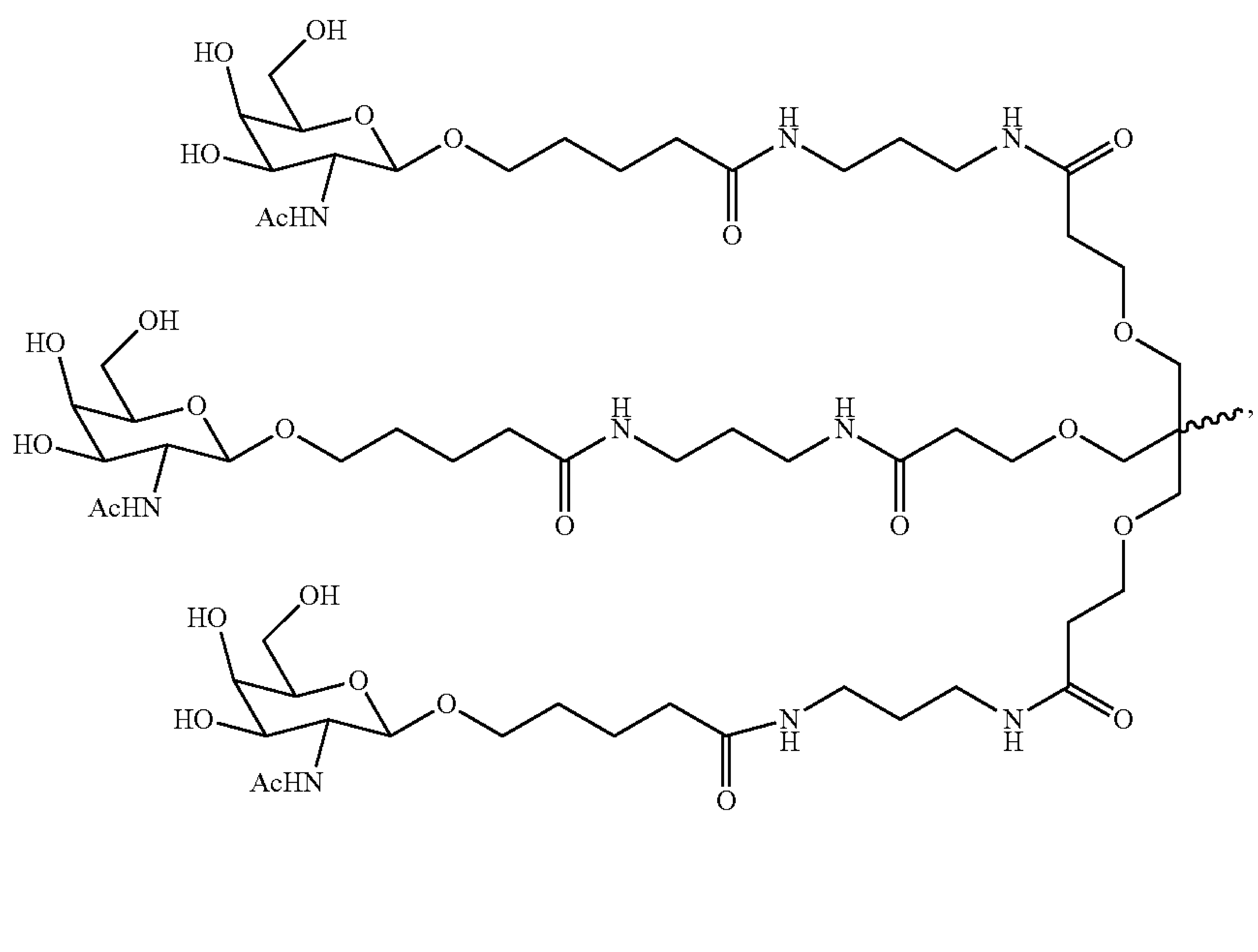
A "cell permeation peptide" is capable of permeating a cell, e.g., a microbial cell, such as a bacterial or fungal cell, or a mammalian cell, such as a human cell. A microbial cell-permeating peptide can be, for example, an α-helical linear peptide (e.g., LL-37 or Ceropin P1), a disulfide bond-containing peptide (e.g., α-defensin, β-defensin or bactenecin), or a peptide containing only one or two dominating amino acids (e.g., PR-39 or indolicidin). A cell permeation peptide can also include a nuclear localization signal (NLS). For example, a cell permeation peptide can be a bipartite amphipathic peptide, such as MPG, which is derived from the fusion peptide domain of HIV-1 gp41 and the NLS of SV40 large T antigen (Simeoni et al., Nucl. Acids Res. 31:2717-2724, 2003).

C. Carbohydrate Conjugates

In some embodiments of the compositions and methods of the invention, an iRNA further comprises a carbohydrate. The carbohydrate conjugated iRNA is advantageous for the in vivo delivery of nucleic acids, as well as compositions suitable for in vivo therapeutic use, as described herein. As used herein, "carbohydrate" refers to a compound which is either a carbohydrate per se made up of one or more monosaccharide units having at least 6 carbon atoms (which can be linear, branched or cyclic) with an oxygen, nitrogen or sulfur atom bonded to each carbon atom; or a compound having as a part thereof a carbohydrate moiety made up of one or more monosaccharide units each having at least six carbon atoms (which can be linear, branched or cyclic), with an oxygen, nitrogen or sulfur atom bonded to each carbon atom. Representative carbohydrates include the sugars (mono-, di-, tri-, and oligosaccharides containing from about 4, 5, 6, 7, 8, or 9 monosaccharide units), and polysaccharides such as starches, glycogen, cellulose and polysaccharide gums. Specific monosaccharides include C5 and above (e.g., C5, C6, C7, or C8) sugars; di- and trisaccharides include sugars having two or three monosaccharide units (e.g., C5, C6, C7, or C8).

In one embodiment, a carbohydrate conjugate for use in the compositions and methods of the invention is a monosaccharide. In another embodiment, a carbohydrate conjugate for use in the compositions and methods of the invention is selected from the group consisting of:

Formula II



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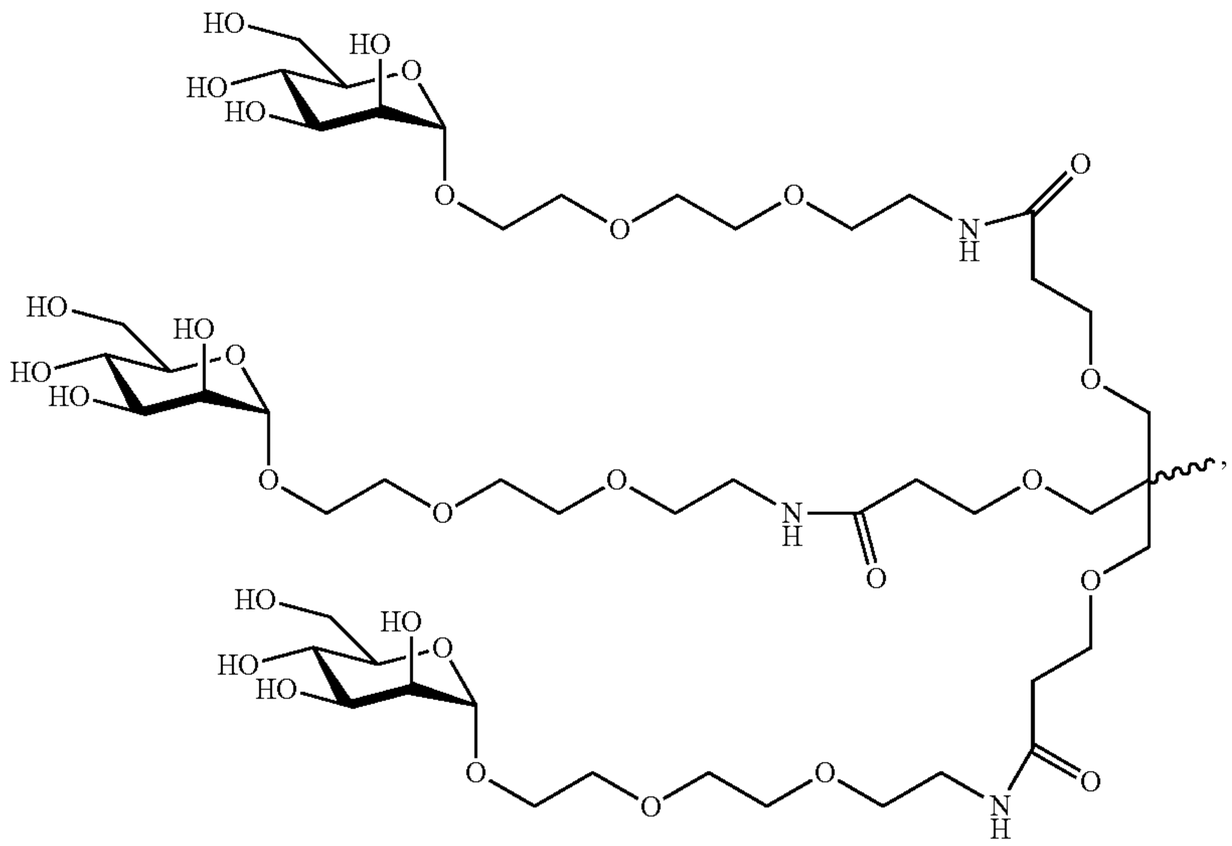
Formula III



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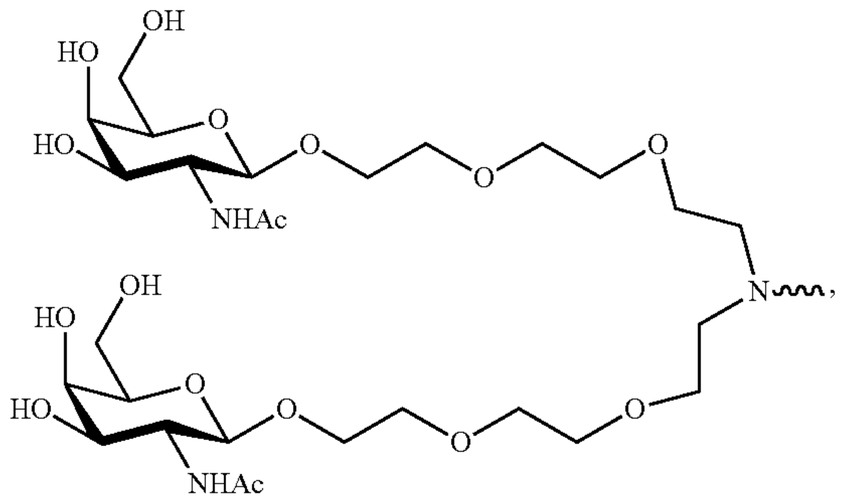

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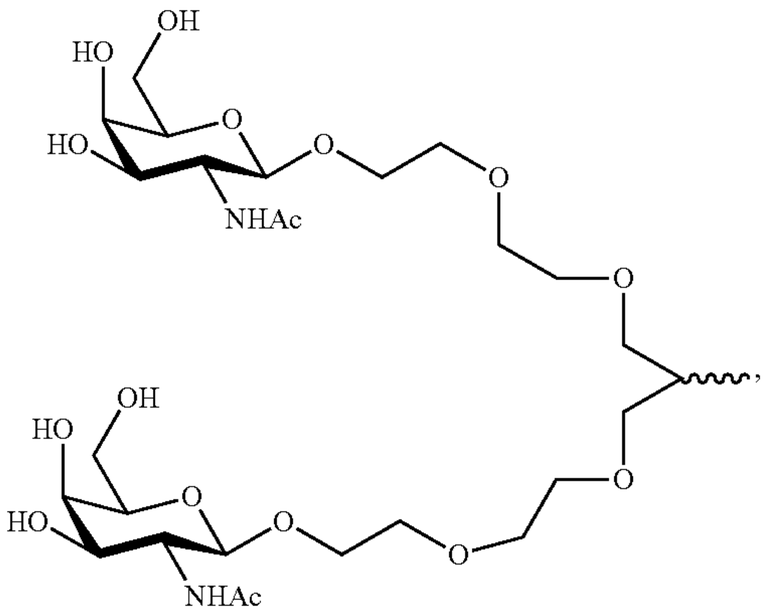

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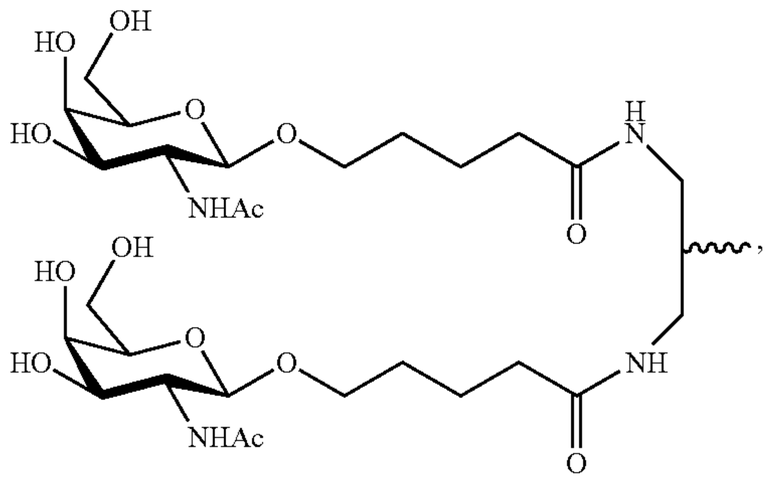

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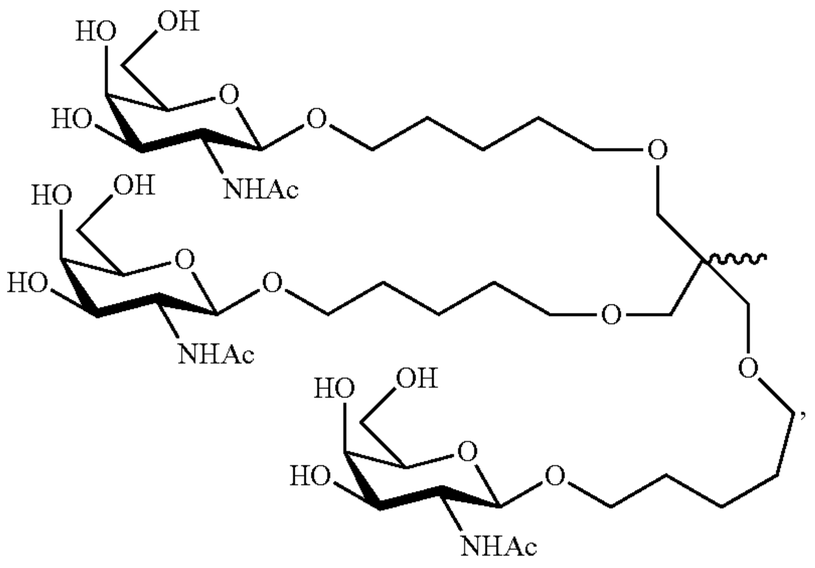

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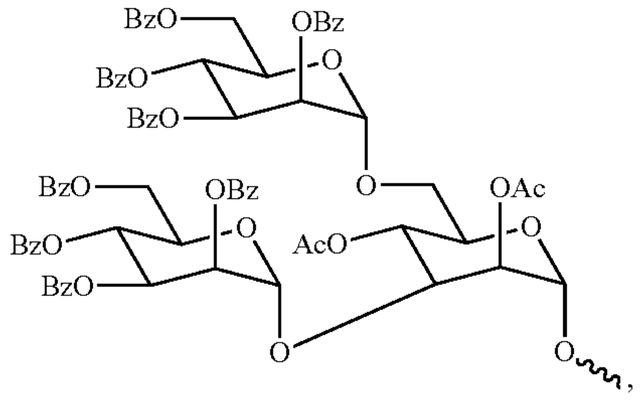

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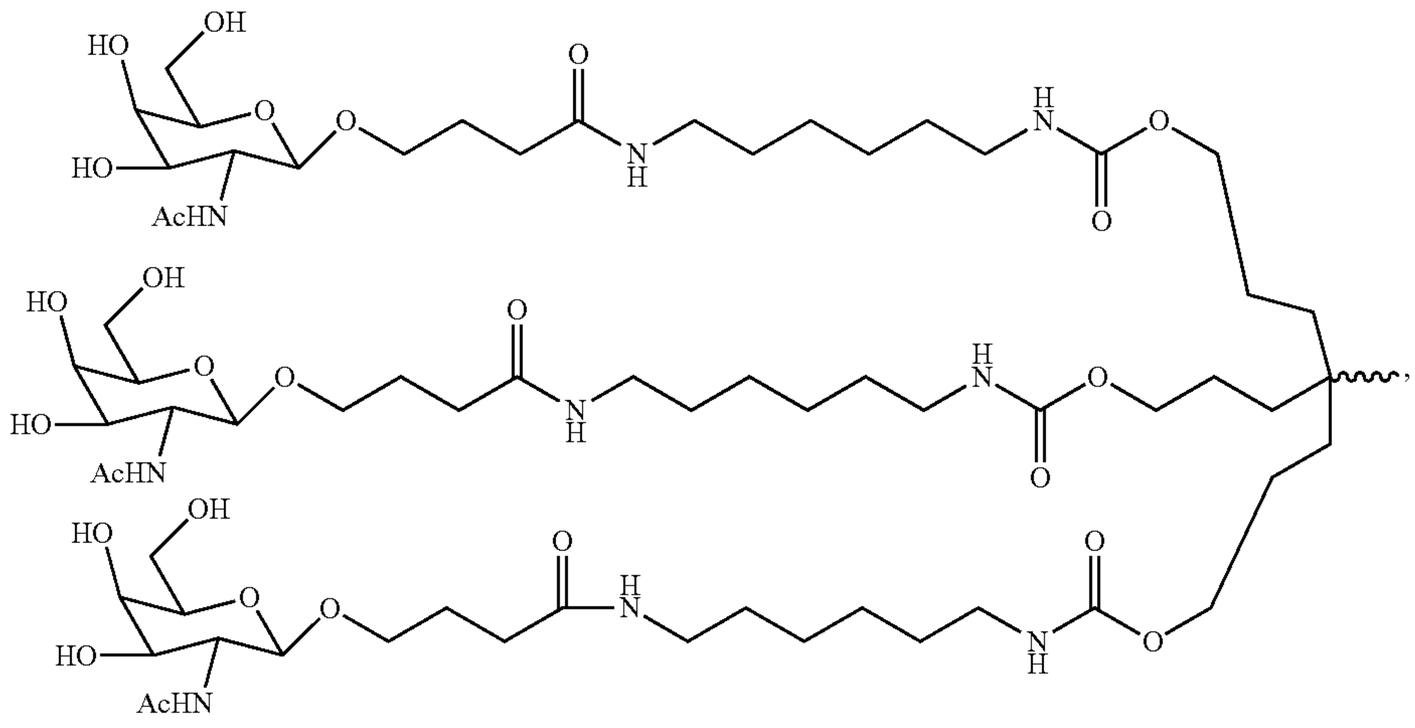

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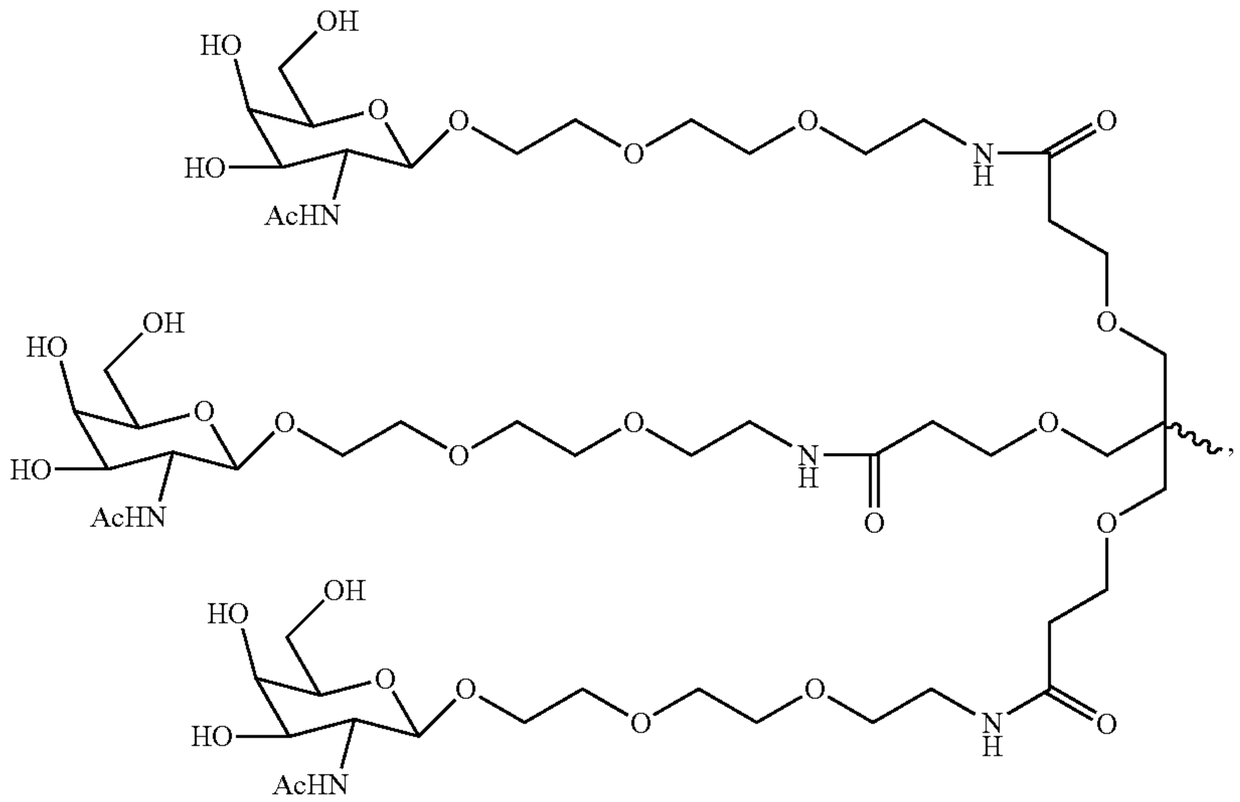

Formula XI

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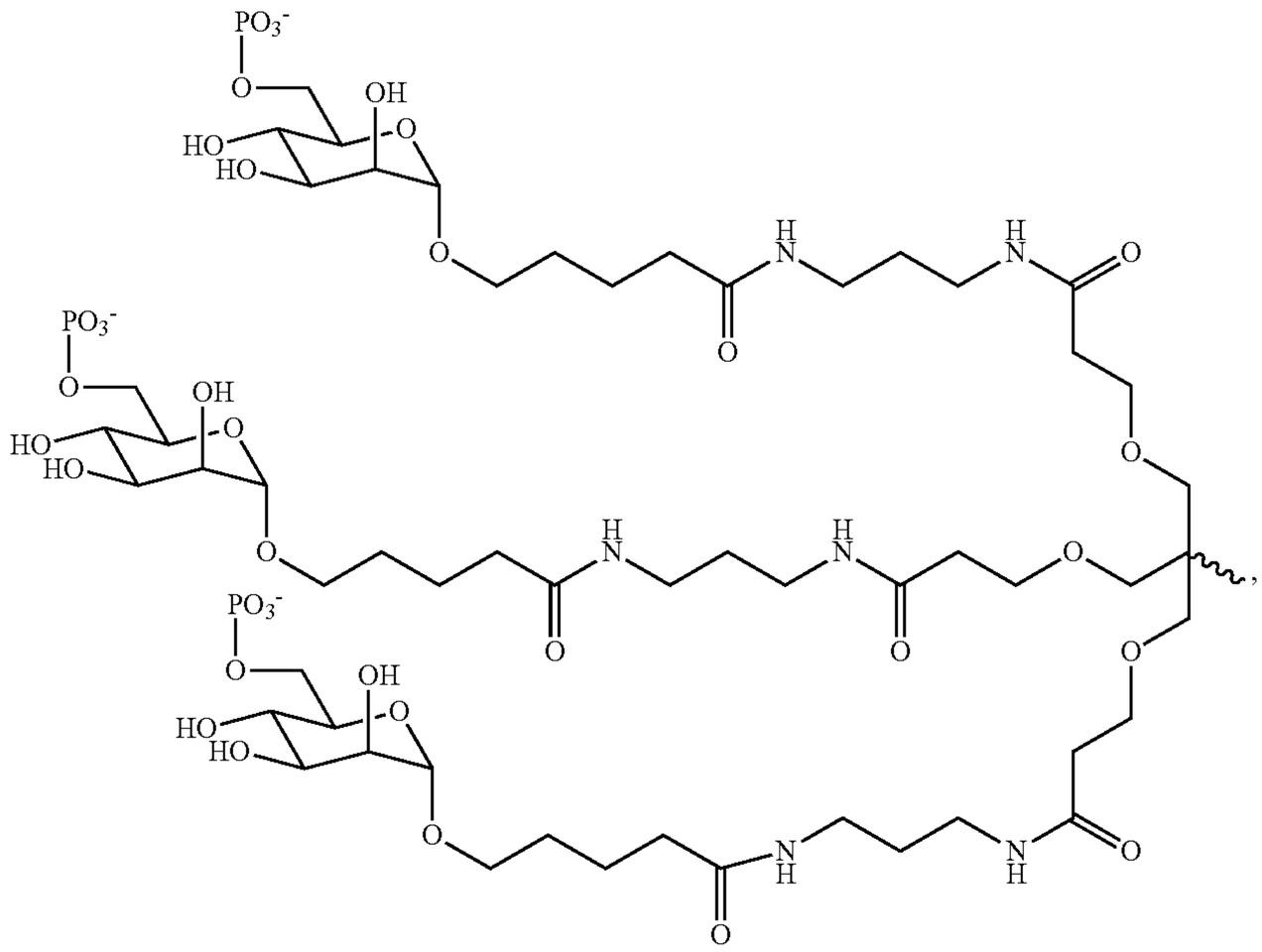
Formula XII



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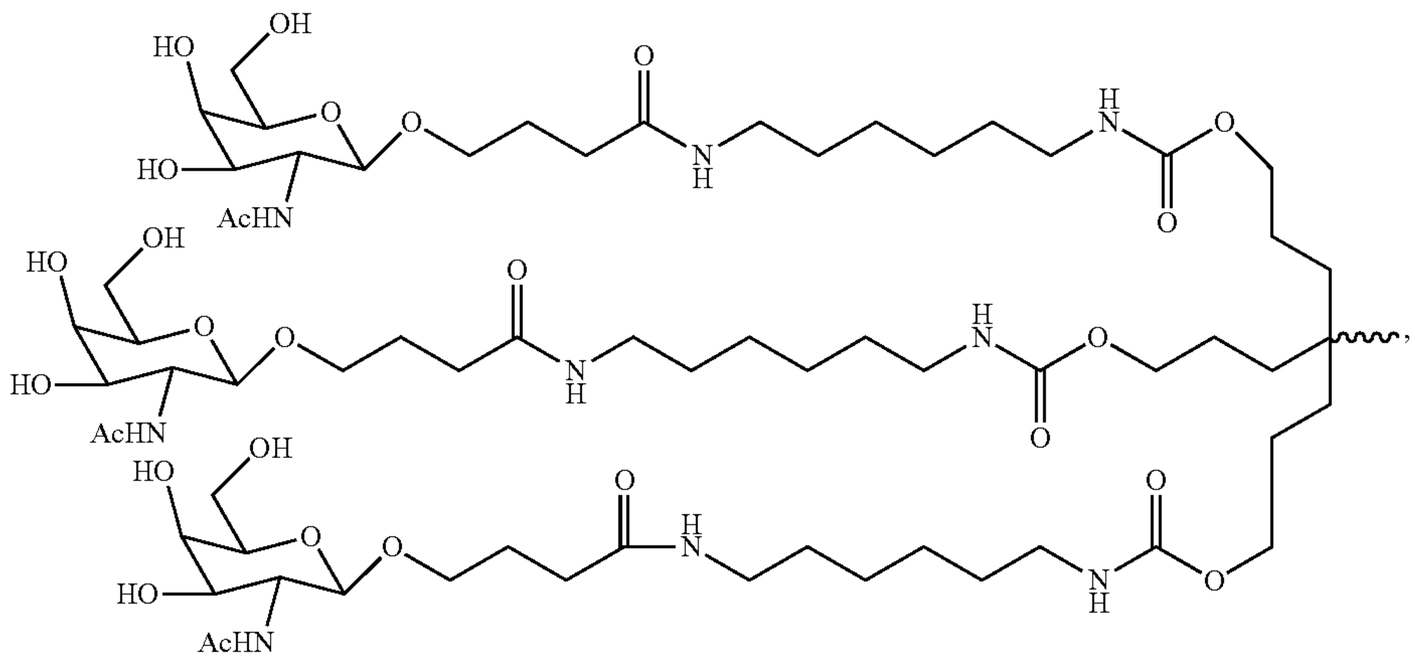
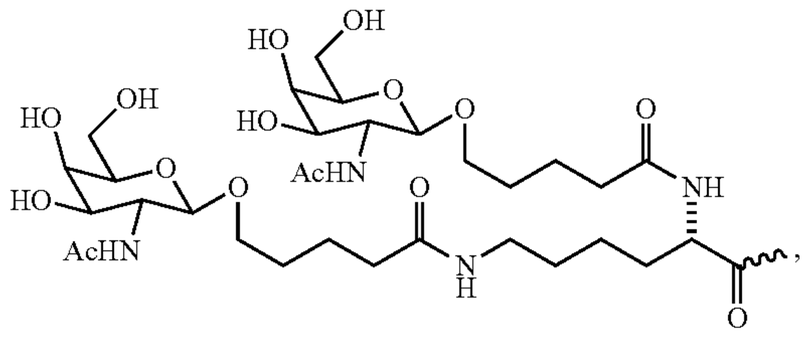


Formula XIV



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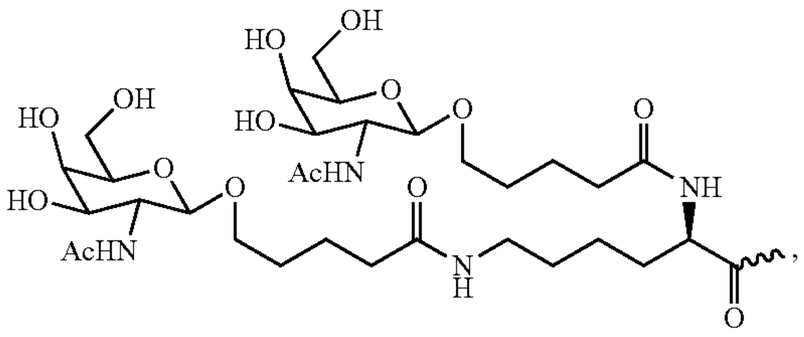

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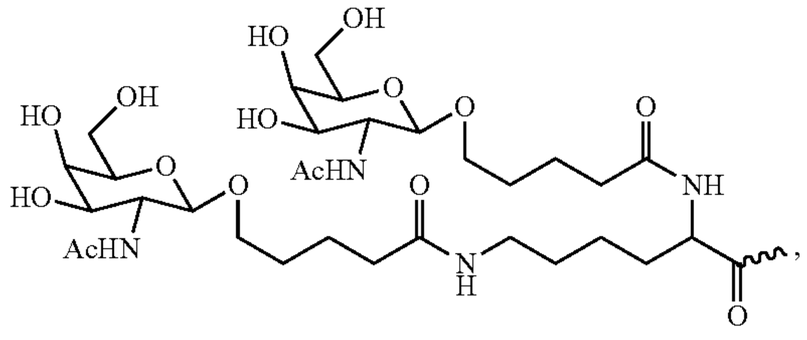
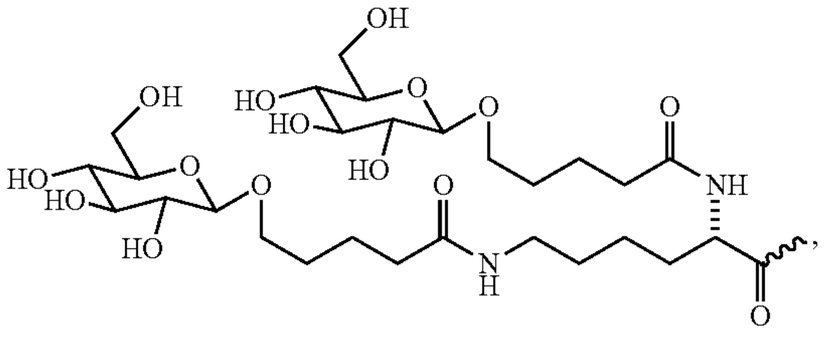
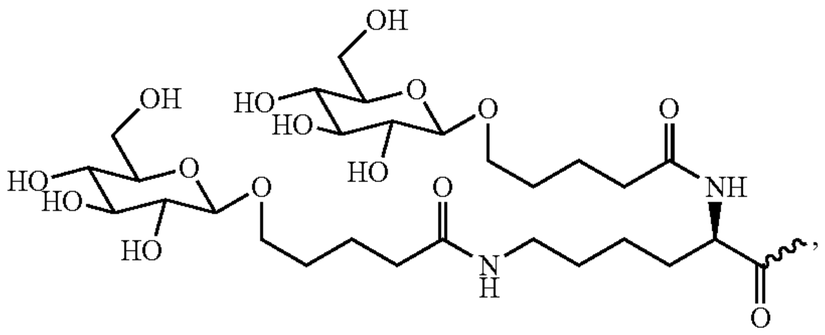


Formula XVII

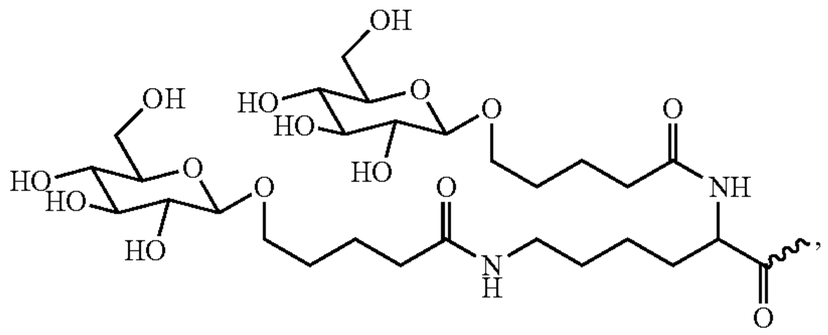


-continued

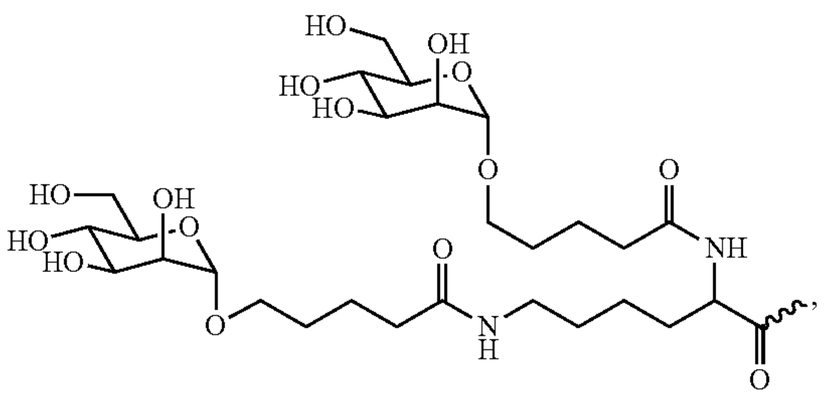
Formula XVIII



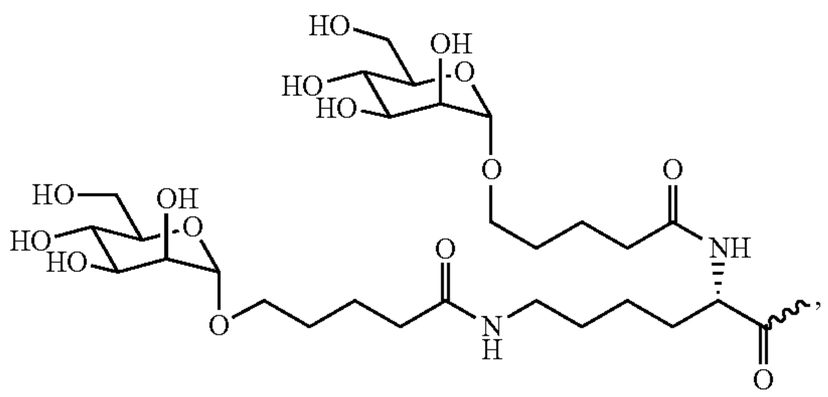
Formula XIX



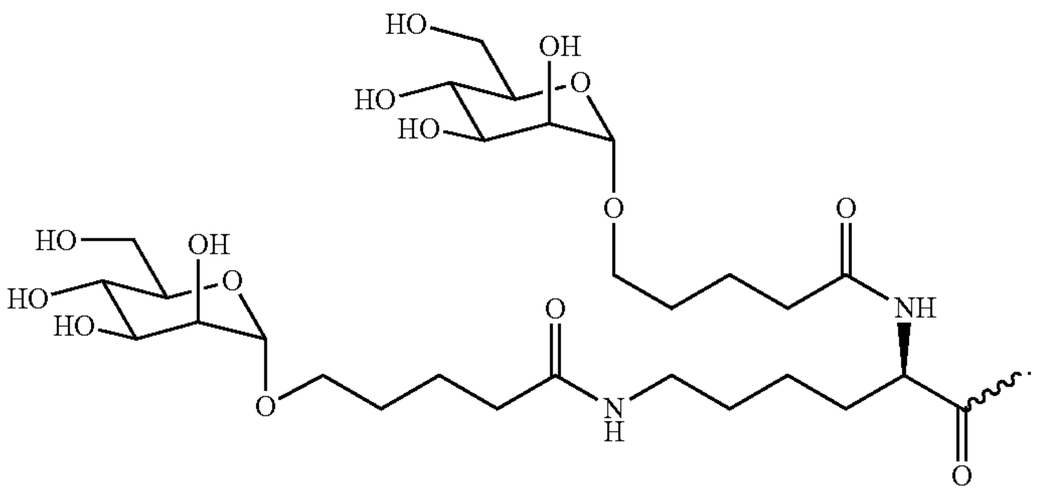
Formula XX



Formula XXI

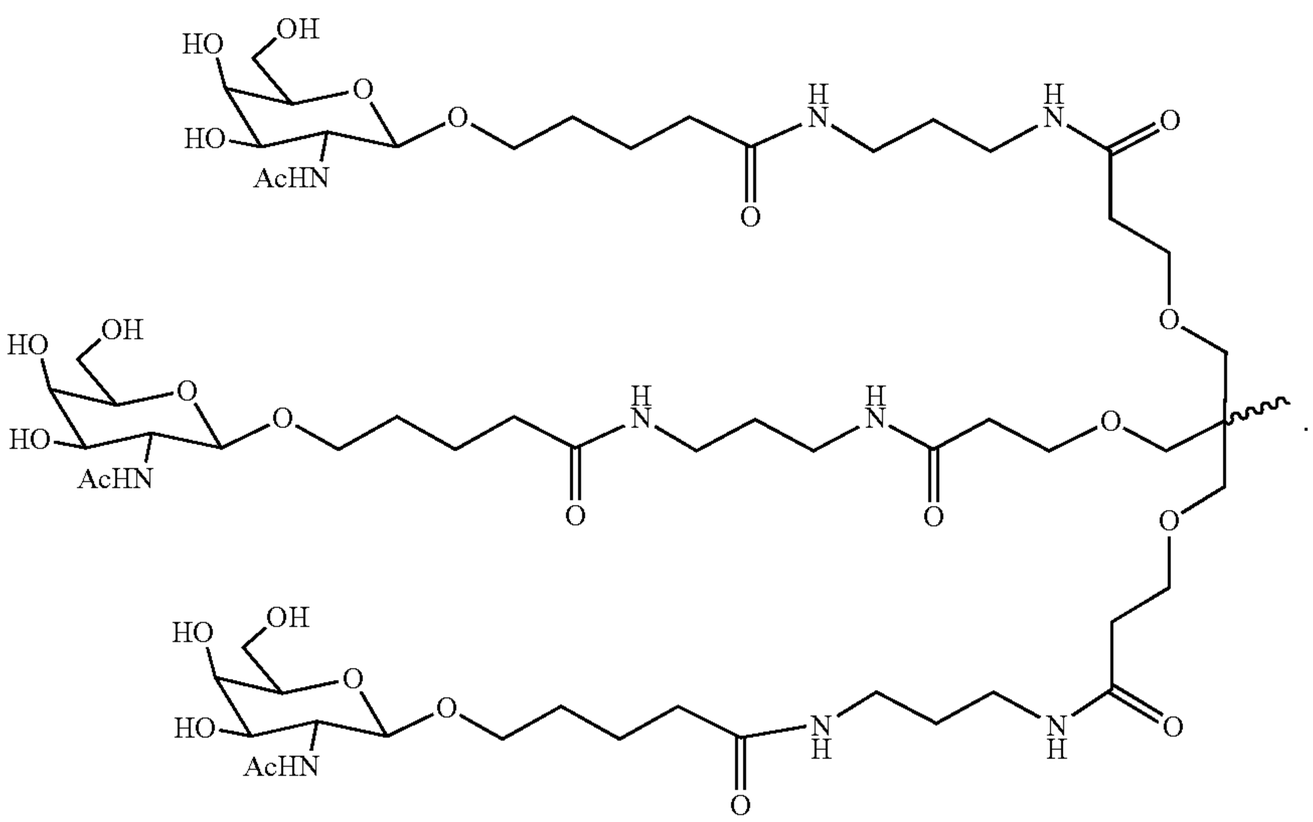


Formula XXII

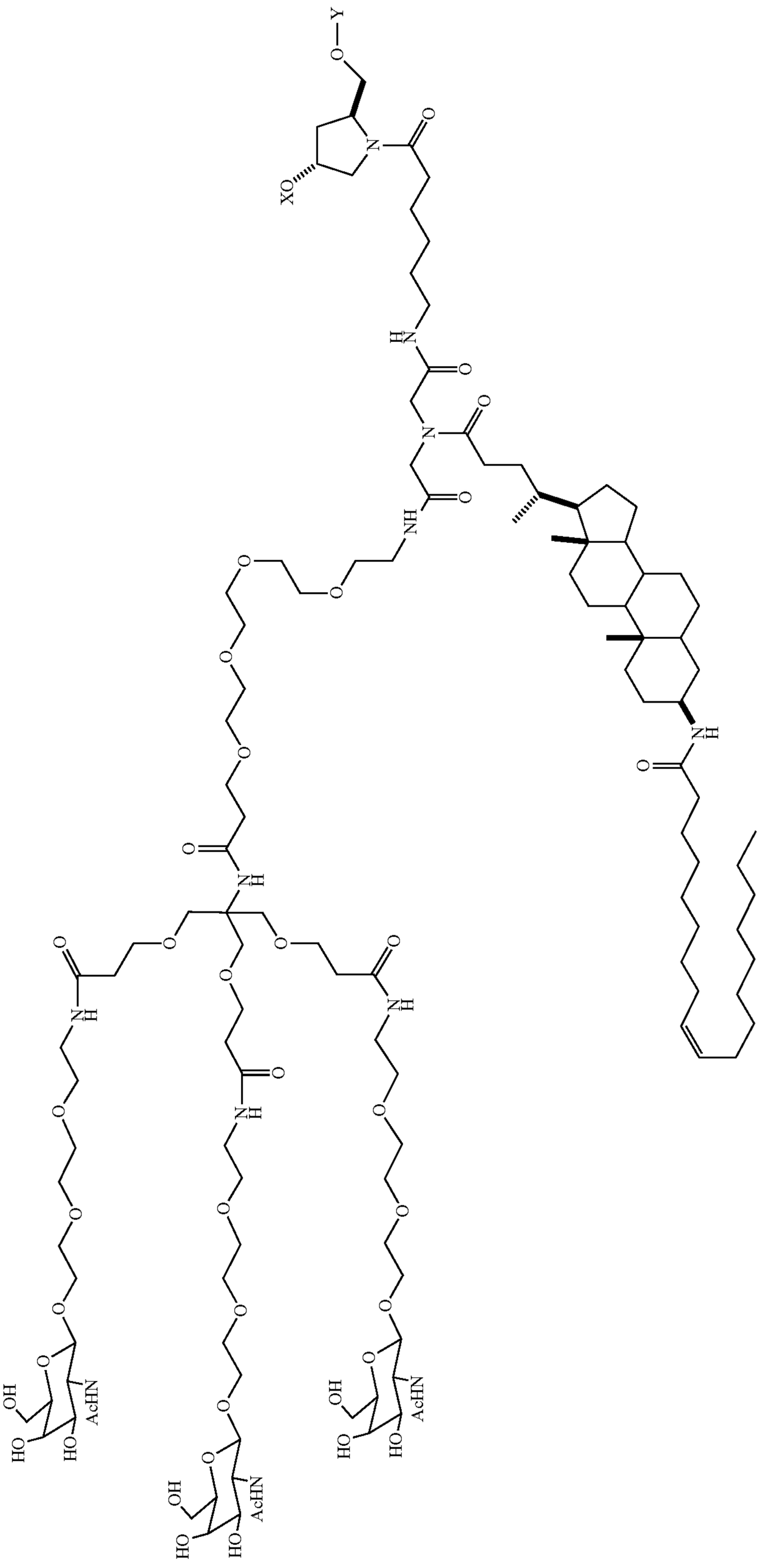


In one embodiment, the monosaccharide is an N-acetyl-galactosamine, such as

Formula II



Another representative carbohydrate conjugate for use in the embodiments described herein includes, but is not limited to,



(Formula XXIII), when one of X or Y is an oligonucleotide, the other is a hydrogen.

In certain embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention via a monovalent linker. In some embodiments, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention via a bivalent linker. In yet other embodiments of the invention, the GalNAc or GalNAc derivative is attached to an iRNA agent of the invention via a trivalent linker.

In one embodiment, the double stranded RNAi agents of the invention comprise one GalNAc or GalNAc derivative attached to the iRNA agent. In another embodiment, the double stranded RNAi agents of the invention comprise a plurality (e.g., 2, 3, 4, 5, or 6) GalNAc or GalNAc derivatives, each independently attached to a plurality of nucleotides of the double stranded RNAi agent through a plurality of monovalent linkers.

In some embodiments, for example, when the two strands of an iRNA agent of the invention are part of one larger molecule connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming a hairpin loop comprising, a plurality of unpaired nucleotides, each unpaired nucleotide within the hairpin loop may independently comprise a GalNAc or GalNAc derivative attached via a monovalent linker. The hairpin loop may also be formed by an extended overhang in one strand of the duplex.

In some embodiments, for example, when the two strands of an iRNA agent of the invention are part of one larger molecule connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming a hairpin loop comprising, a plurality of unpaired nucleotides, each unpaired nucleotide within the hairpin loop may independently comprise a GalNAc or GalNAc derivative attached via a monovalent linker. The hairpin loop may also be formed by an extended overhang in one strand of the duplex.

In some embodiments, the carbohydrate conjugate further comprises one or more additional ligands as described above, such as, but not limited to, a PK modulator or a cell permeation peptide.

Additional carbohydrate conjugates suitable for use in the present invention include those described in PCT Publication Nos. WO 2014/179620 and WO 2014/179627, the entire contents of each of which are incorporated herein by reference.

D. Linkers

In some embodiments, the conjugate or ligand described herein can be attached to an iRNA oligonucleotide with various linkers that can be cleavable or non-cleavable.

The term "linker" or "linking group" means an organic moiety that connects two parts of a compound, e.g., covalently attaches two parts of a compound. Linkers typically comprise a direct bond or an atom such as oxygen or sulfur, a unit such as NR8, C(O), C(O)NH, SO, SO_2, SO_2NH or a chain of atoms, such as, but not limited to, substituted or unsubstituted alkyl, substituted or unsubstituted alkenyl, substituted or unsubstituted alkynyl, arylalkyl, arylalkenyl, arylalkynyl, heteroarylalkyl, heteroarylalkenyl, heteroarylalkynyl, heterocyclylalkyl, heterocyclylalkenyl, heterocyclylalkynyl, aryl, heteroaryl, heterocyclyl, cycloalkyl, cycloalkenyl, alkylarylalkyl, alkylarylalkenyl, alkylarylalkynyl, alkenylarylalkyl, alkenylarylalkenyl, alkenylarylalkynyl, alkynylarylalkyl, alkynylarylalkenyl, alkynylarylalkynyl, alkylheteroarylalkyl, alkylheteroarylalkenyl, alkylheteroarylalkynyl, alkenylheteroarylalkyl, alkenylheteroarylalkenyl, alkenylheteroarylalkynyl, alkynylheteroarylalkyl, alkynylheteroarylalkenyl, alkynylheteroarylalkynyl, alkylheterocyclylalkyl, alkylheterocyclylalkenyl, alkylhererocyclylalkynyl, alkenylheterocyclylalkyl, alkenylheterocyclylalkenyl, alkenylheterocyclylalkynyl, alkynylheterocyclylalkyl, alkynylheterocyclylalkenyl, alkynylheterocyclylalkynyl, alkylaryl, alkenylaryl, alkynylaryl, alkylheteroaryl, alkenylheteroaryl, alkynylhereroaryl, which one or more methylenes can be interrupted or terminated by O, S, S(O), SO_2, N(R8), C(O), substituted or unsubstituted aryl, substituted or unsubstituted heteroaryl, or substituted or unsubstituted heterocyclic; where R8 is hydrogen, acyl, aliphatic, or substituted aliphatic. In one embodiment, the linker is about 1-24 atoms, 2-24, 3-24, 4-24, 5-24, 6-24, 6-18, 7-18, 8-18, 7-17, 8-17, 6-16, 7-16, or 8-16 atoms.

A cleavable linking group is one which is sufficiently stable outside the cell, but which upon entry into a target cell is cleaved to release the two parts the linker is holding together. In a preferred embodiment, the cleavable linking group is cleaved at least 10 times, 20, times, 30 times, 40 times, 50 times, 60 times, 70 times, 80 times, 90 times, or 100 times faster in a target cell or under a first reference condition (which can, e.g., be selected to mimic or represent intracellular conditions) than in the blood of a subject, or under a second reference condition (which can, e.g., be selected to mimic or represent conditions found in the blood or serum).

Cleavable linking groups are susceptible to cleavage agents, e.g., pH, redox potential, or the presence of degradative molecules. Generally, cleavage agents are more prevalent or found at higher levels or activities inside cells than in serum or blood. Examples of such degradative agents include: redox agents which are selected for particular substrates or which have no substrate specificity, including, e.g., oxidative or reductive enzymes or reductive agents such as mercaptans, present in cells, that can degrade a redox cleavable linking group by reduction; esterases; endosomes or agents that can create an acidic environment, e.g., those that result in a pH of five or lower; enzymes that can hydrolyze or degrade an acid cleavable linking group by acting as a general acid, peptidases (which can be substrate specific), and phosphatases.

A cleavable linkage group, such as a disulfide bond can be susceptible to pH. The pH of human serum is 7.4, while the average intracellular pH is slightly lower, ranging from about 7.1-7.3. Endosomes have a more acidic pH, in the range of 5.5-6.0, and lysosomes have an even more acidic pH at around 5.0. Some linkers will have a cleavable linking group that is cleaved at a preferred pH, thereby releasing a cationic lipid from the ligand inside the cell, or into the desired compartment of the cell.

A linker can include a cleavable linking group that is cleavable by a particular enzyme. The type of cleavable linking group incorporated into a linker can depend on the cell to be targeted. For example, a liver-targeting ligand can be linked to a cationic lipid through a linker that includes an ester group. Liver cells are rich in esterases, and therefore the linker will be cleaved more efficiently in liver cells than in cell types that are not esterase-rich. Other cell-types rich in esterases include cells of the lung, renal cortex, and testis.

Linkers that contain peptide bonds can be used when targeting cell types rich in peptidases, such as liver cells and synoviocytes.

In general, the suitability of a candidate cleavable linking group can be evaluated by testing the ability of a degradative agent (or condition) to cleave the candidate linking group. It

will also be desirable to also test the candidate cleavable linking group for the ability to resist cleavage in the blood or when in contact with other non-target tissue. Thus, one can determine the relative susceptibility to cleavage between a first and a second condition, where the first is selected to be indicative of cleavage in a target cell and the second is selected to be indicative of cleavage in other tissues or biological fluids, e.g., blood or serum. The evaluations can be carried out in cell free systems, in cells, in cell culture, in organ or tissue culture, or in whole animals. It can be useful to make initial evaluations in cell-free or culture conditions and to confirm by further evaluations in whole animals. In preferred embodiments, useful candidate compounds are cleaved at least 2, 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100 times faster in the cell (or under in vitro conditions selected to mimic intracellular conditions) as compared to blood or serum (or under in vitro conditions selected to mimic extracellular conditions).

i. Redox Cleavable Linking Groups

In certain embodiments, a cleavable linking group is a redox cleavable linking group that is cleaved upon reduction or oxidation. An example of reductively cleavable linking group is a disulphide linking group (—S—S—). To determine if a candidate cleavable linking group is a suitable "reductively cleavable linking group," or for example is suitable for use with a particular iRNA moiety and particular targeting agent one can look to methods described herein. For example, a candidate can be evaluated by incubation with dithiothreitol (DTT), or other reducing agent using reagents know in the art, which mimic the rate of cleavage which would be observed in a cell, e.g., a target cell. The candidates can also be evaluated under conditions which are selected to mimic blood or serum conditions. In one, candidate compounds are cleaved by at most about 10% in the blood. In other embodiments, useful candidate compounds are degraded at least about 2, 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, or about 100 times faster in the cell (or under in vitro conditions selected to mimic intracellular conditions) as compared to blood (or under in vitro conditions selected to mimic extracellular conditions). The rate of cleavage of candidate compounds can be determined using standard enzyme kinetics assays under conditions chosen to mimic intracellular media and compared to conditions chosen to mimic extracellular media.

ii. Phosphate-Based Cleavable Linking Groups

In other embodiments, a cleavable linker comprises a phosphate-based cleavable linking group. A phosphate-based cleavable linking group is cleaved by agents that degrade or hydrolyze the phosphate group. An example of an agent that cleaves phosphate groups in cells are enzymes such as phosphatases in cells. Examples of phosphate-based linking groups are —O—P(O)(ORk)-O—, —O—P(S)(ORk)-O—, —O—P(S)(SRk)-O—, —S—P(O)(ORk)-O—, —O—P(O)(ORk)-S—, —S—P(O)(ORk)-S—, —O—P(S)(ORk)-S—, —S—P(S)(ORk)-O—, —O—P(O)(Rk)-O—, —O—P(S)(Rk)-O—, —S—P(O)(Rk)-O—, —S—P(S)(Rk)-O—, —S—P(O)(Rk)-S—, —O—P(S)(Rk)-S—. Preferred embodiments are —O—P(O)(OH)—O—, —O—P(S)(OH)—O—, —O—P(S)(SH)—O—, —S—P(O)(OH)—O—, —O—P(O)(OH)—S—, —S—P(O)(OH)—S—, —O—P(S)(OH)—S—, —S—P(S)(OH)—O—, —O—P(O)(H)—O—, —O—P(S)(H)—O—, —S—P(O)(H)—O, —S—P(S)(H)—O—, —S—P(O)(H)—S—, and —O—P(S)(H)—S—. A preferred embodiment is —O—P(O)(OH)—O—. These candidates can be evaluated using methods analogous to those described above.

iii. Acid cleavable linking groups

In other embodiments, a cleavable linker comprises an acid cleavable linking group. An acid cleavable linking group is a linking group that is cleaved under acidic conditions. In preferred embodiments acid cleavable linking groups are cleaved in an acidic environment with a pH of about 6.5 or lower (e.g., about 6.0, 5.5, 5.0, or lower), or by agents such as enzymes that can act as a general acid. In a cell, specific low pH organelles, such as endosomes and lysosomes can provide a cleaving environment for acid cleavable linking groups. Examples of acid cleavable linking groups include but are not limited to hydrazones, esters, and esters of amino acids. Acid cleavable groups can have the general formula —C=NN—, C(O)O, or —OC(O). A preferred embodiment is when the carbon attached to the oxygen of the ester (the alkoxy group) is an aryl group, substituted alkyl group, or tertiary alkyl group such as dimethyl pentyl or t-butyl. These candidates can be evaluated using methods analogous to those described above.

iv. Ester-Based Linking Groups

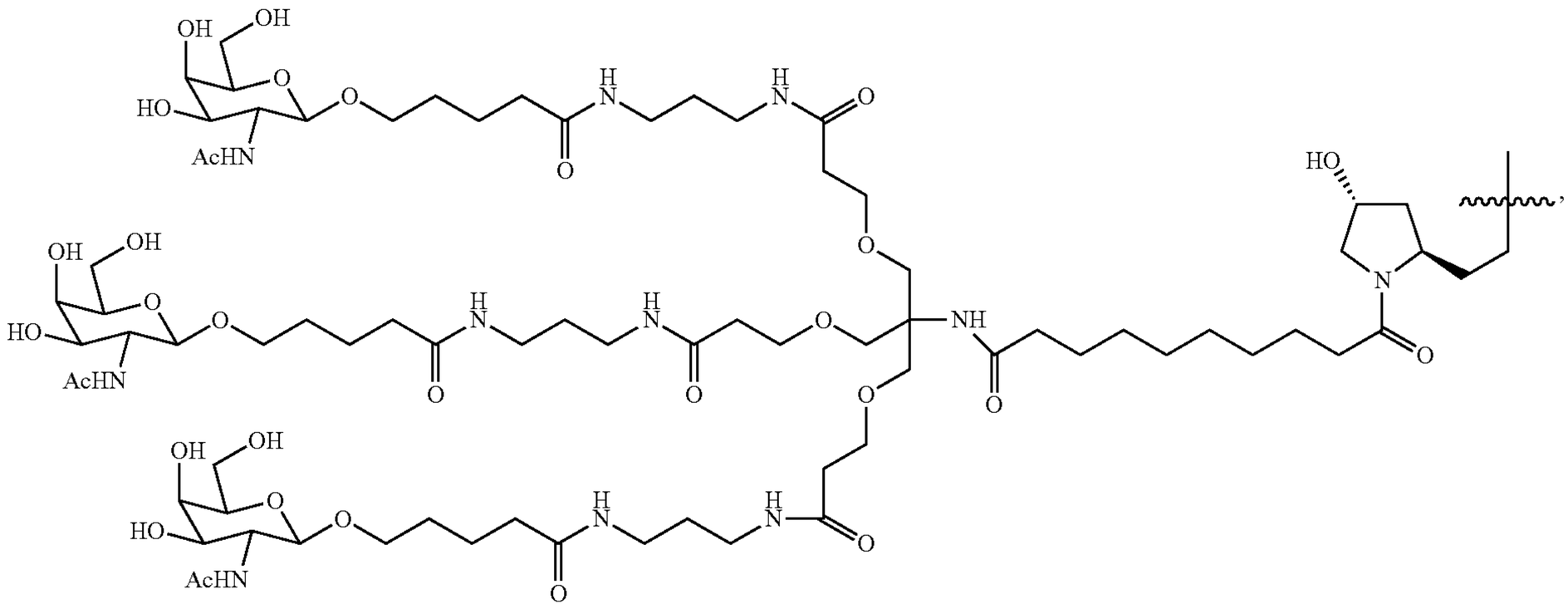
In other embodiments, a cleavable linker comprises an ester-based cleavable linking group. An ester-based cleavable linking group is cleaved by enzymes such as esterases and amidases in cells. Examples of ester-based cleavable linking groups include, but are not limited to, esters of alkylene, alkenylene and alkynylene groups. Ester cleavable linking groups have the general formula —C(O)O—, or —OC(O)—. These candidates can be evaluated using methods analogous to those described above.

v. Peptide-Based Cleaving Groups

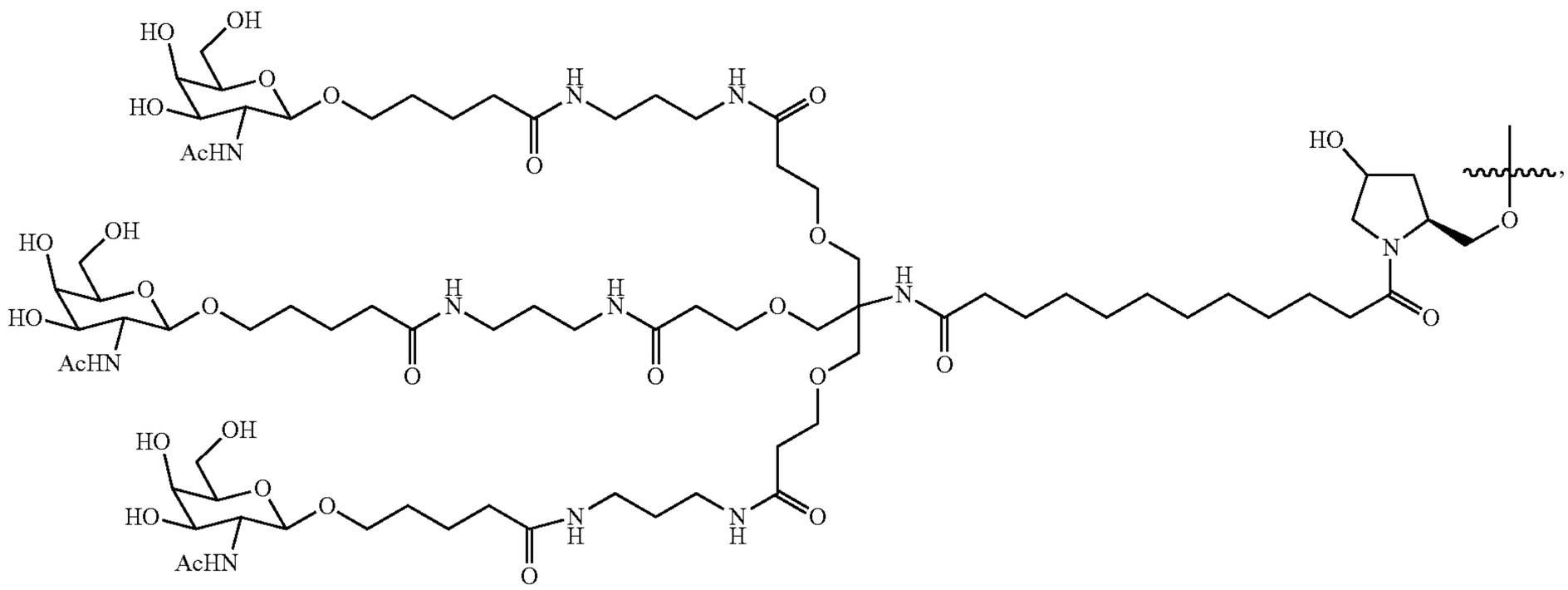
In yet other embodiments, a cleavable linker comprises a peptide-based cleavable linking group. A peptide-based cleavable linking group is cleaved by enzymes such as peptidases and proteases in cells. Peptide-based cleavable linking groups are peptide bonds formed between amino acids to yield oligopeptides (e.g., dipeptides, tripeptides etc.) and polypeptides. Peptide-based cleavable groups do not include the amide group (—C(O)NH—). The amide group can be formed between any alkylene, alkenylene or alkynelene. A peptide bond is a special type of amide bond formed between amino acids to yield peptides and proteins. The peptide based cleavage group is generally limited to the peptide bond (i.e., the amide bond) formed between amino acids yielding peptides and proteins and does not include the entire amide functional group. Peptide-based cleavable linking groups have the general formula —NHCHRAC(O)NHCHRBC(O)—, where RA and RB are the R groups of the two adjacent amino acids. These candidates can be evaluated using methods analogous to those described above.

In some embodiments, an iRNA of the invention is conjugated to a carbohydrate through a linker. Non-limiting examples of iRNA carbohydrate conjugates with linkers of the compositions and methods of the invention include, but are not limited to,

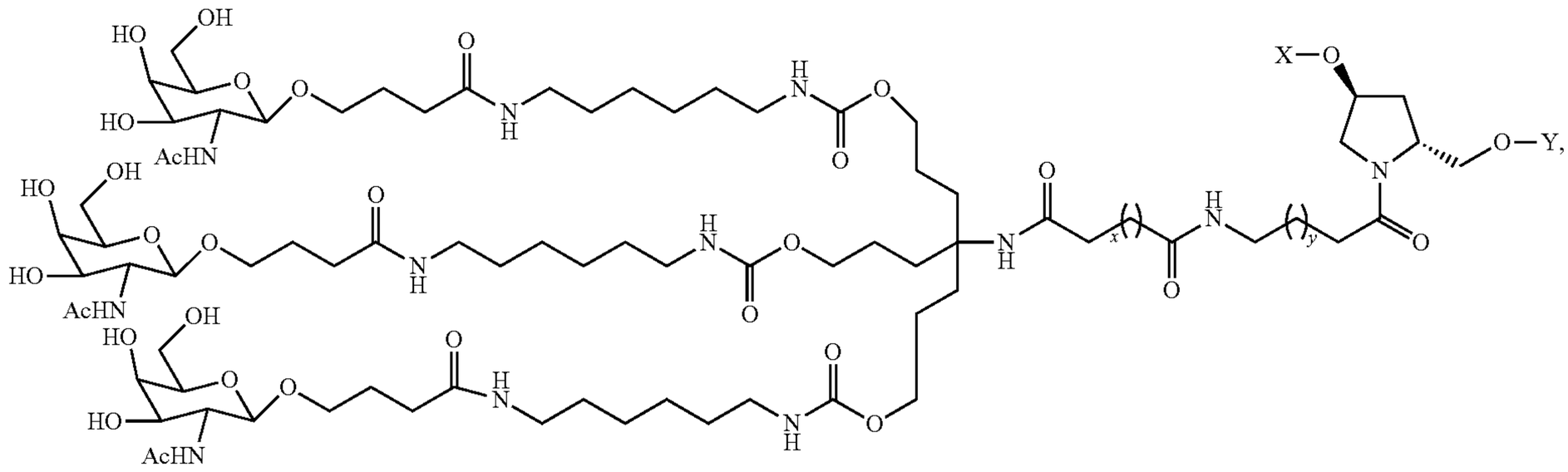
(Formula XXIV)



(Formula XXV)



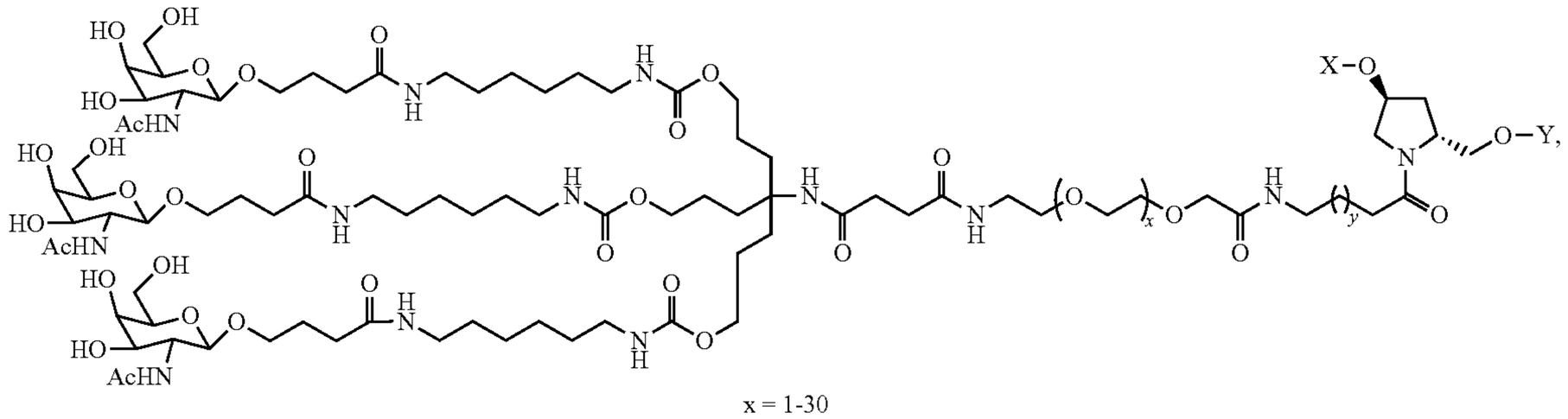
(Formula XXVI)



x = 1-30
y = 1-15

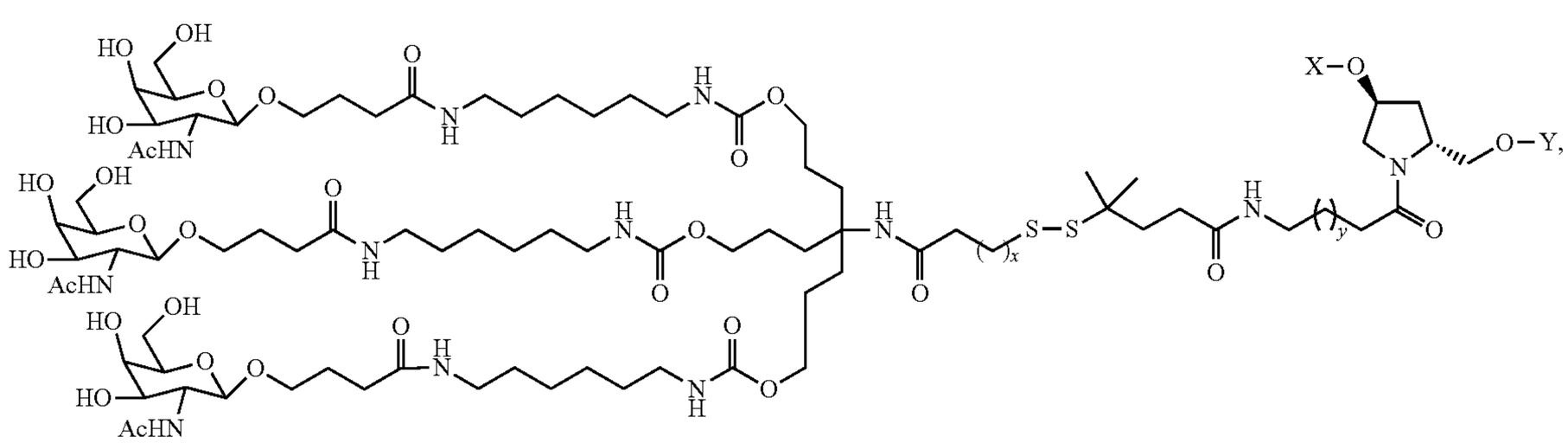
-continued

(Formula XXVII)



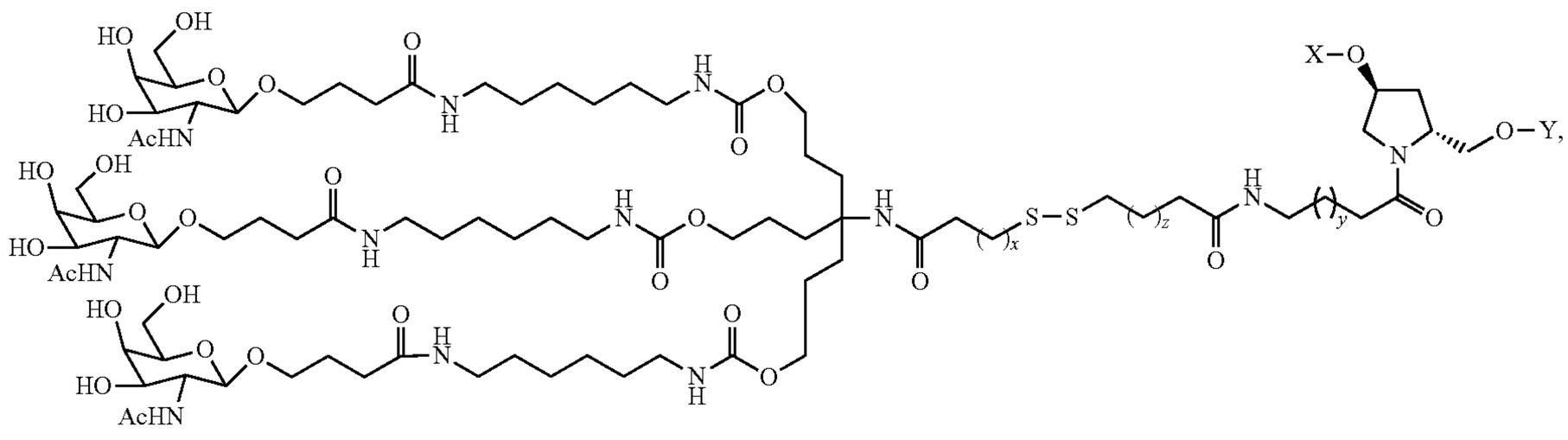
x = 1-30
y = 1-15

(Formula XXVIII)



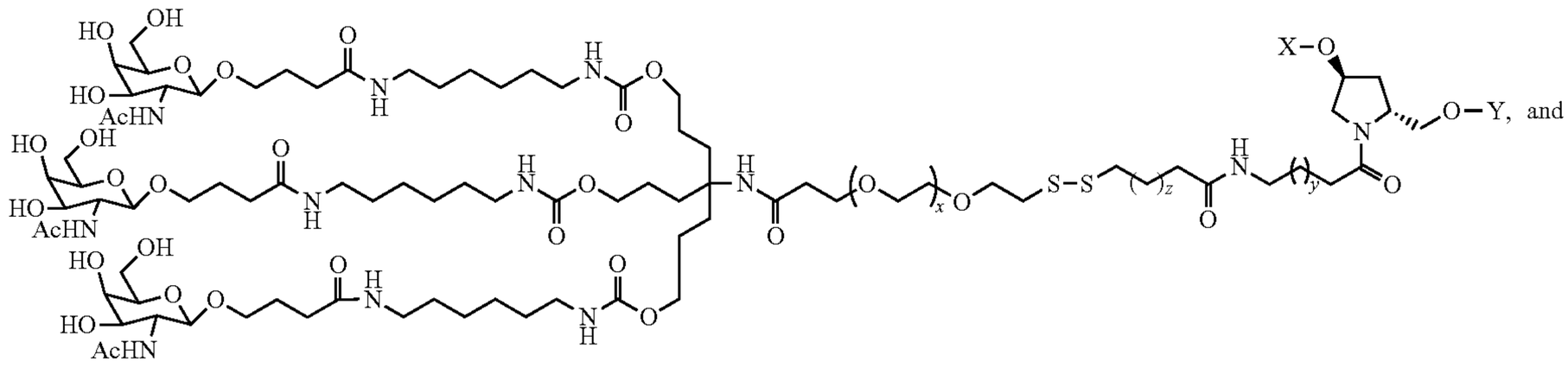
x = 0-30
y = 1-15

(Formula XXIX)



x = 0-30
y = 1-15
z = 1-20

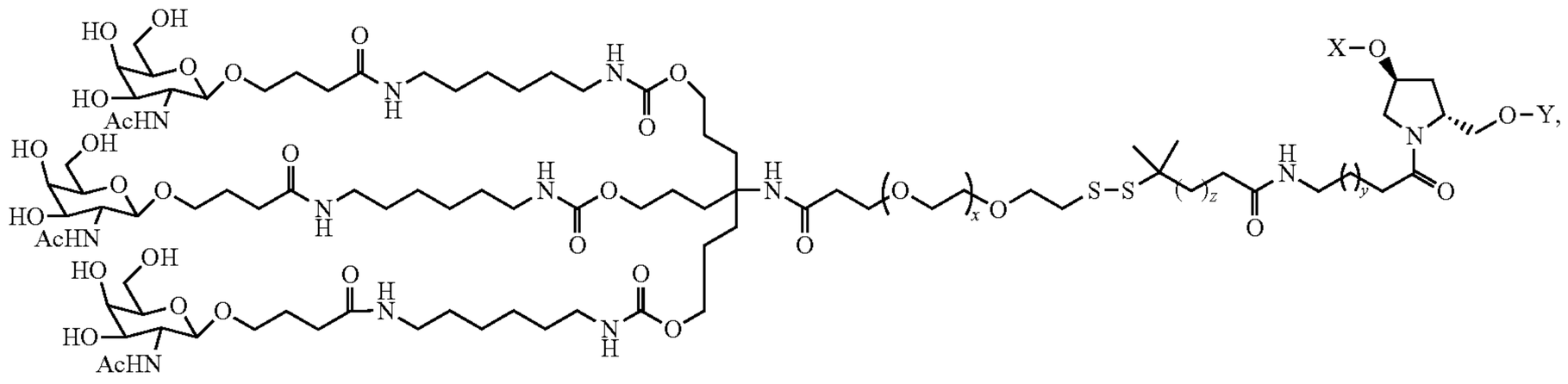
(Formula XXX)



x = 1-30
y = 1-15
z = 1-20

-continued

(Formula XXXI)



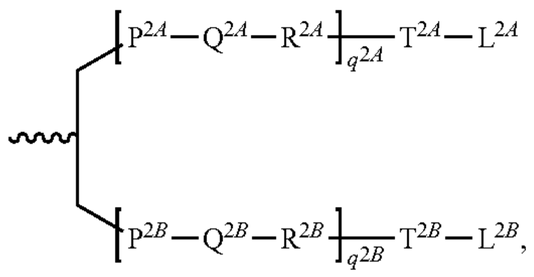
x = 1-30
y = 1-15
z = 1-20

when one of X or Y is an oligonucleotide, the other is a hydrogen.

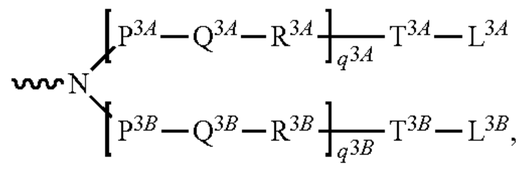
In certain embodiments of the compositions and methods of the invention, a ligand is one or more "GalNAc" (N-acetylgalactosamine) derivatives attached through a bivalent or trivalent branched linker.

In certain embodiments, a dsRNA of the invention is conjugated to a bivalent or trivalent branched linker selected from the group of structures shown in any of formula (XXXII)-(XXXV):

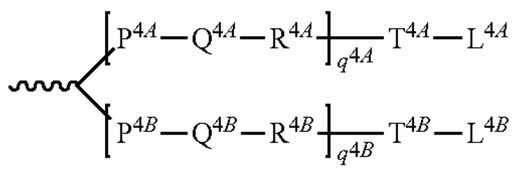
Formula XXXII



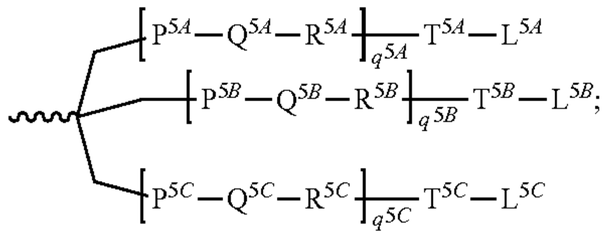
Formula XXXIII



Formula XXXIV



Formula XXXV



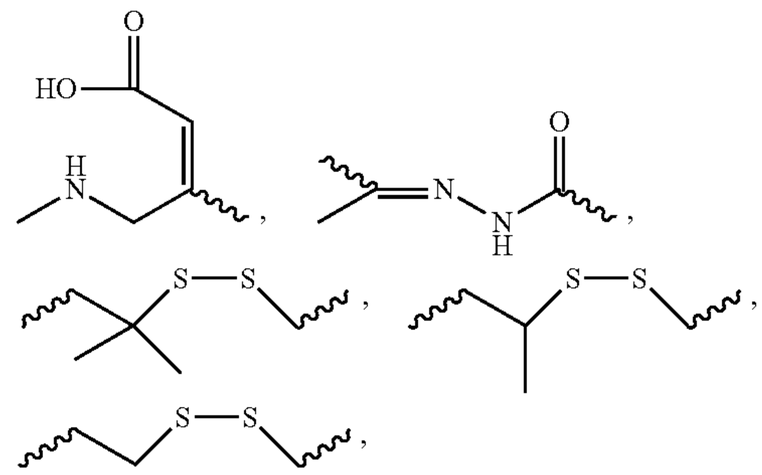
wherein:

q2A, q2B, q3A, q3B, q4A, q4B, q5A, q5B and q5C represent independently for each occurrence 0-20 and wherein the repeating unit can be the same or different;

P^{2A}, P^{2B}, P^{3A}, P^{3B}, P^{4A}, P^{4B}, P^{5A}, P^{5B}, P^{5C}, T^{2A}, T^{2B}, T^{3A}, T^{3B}, T^{4A}, T^{4B}, T^{4A}, T^{5B}, T^{5C} are each independently for each occurrence absent, CO, NH, O, S, OC(O), NHC(O), CH_2, CH_2NH, or CH_2O;

Q^{2A}, Q^{2B}, Q^{3A}, Q^{3B}, Q^{4A}, Q^{4B}, Q^{5A}, Q^{5B}, Q^{5C} are independently for each occurrence absent, alkylene, substituted alkylene wherein one or more methylenes can be interrupted or terminated by one or more of O, S, S(O), SO_2, $N(R^N)$, C(R')=C(R"), C≡C, or C(O);

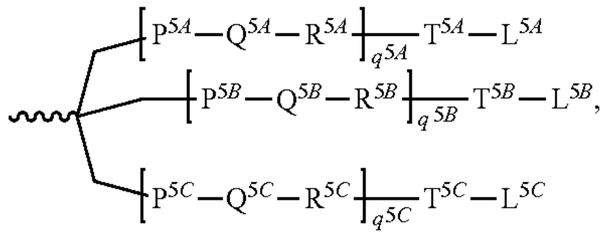
R^{2A}, R^{2B}, R^{3A}, R^{3B}, R^{4A}, R^{4B}, R^{5A}, R^{5B}, R^{5C} are each independently for each occurrence absent, NH, O, S, CH_2, C(O)O, C(O)NH, $NHCH(R^a)C(O)$, —C(O)—$CH(R^a)$—NH—, CO, CH=N—O,



or heterocyclyl;

L^{2A}, L^{2B}, L^{3A}, L^{3B}, L^{4A}, L^{4B}, L^{5A}, L^{5B}, and L^{5C} represent the ligand; i.e. each independently for each occurrence a monosaccharide (such as GalNAc), disaccharide, trisaccharide, tetrasaccharide, oligosaccharide, or polysaccharide; and R^a is H or amino acid side chain. Trivalent conjugating GalNAc derivatives are particularly useful for use with RNAi agents for inhibiting the expression of a target gene, such as those of formula (XXXV):

Formula XXXV



wherein L^{5A}, L^{5B} and L^{5C} represent a monosaccharide, such as GalNAc derivative.

Examples of suitable bivalent and trivalent branched linker groups conjugating GalNAc derivatives include, but are not limited to, the structures recited above as formulas II, VII, XI, X, and XIII.

Representative US patents that teach the preparation of RNA conjugates include, but are not limited to, U.S. Pat. Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,

313; 5,545,730; 5,552,538; 5,578,717, 5,580,731; 5,591, 584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486, 603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605, 735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835, 263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112, 963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245, 022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292, 873; 5,317,098; 5,371,241, 5,391,723; 5,416,203, 5,451, 463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567, 810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597, 696; 5,599,923; 5,599,928; 5,688,941; 6,294,664; 6,320, 017; 6,576,752; 6,783,931; 6,900,297; 7,037,646; and 8,106,022, the entire contents of each of which are hereby incorporated herein by reference.

It is not necessary for all positions in a given compound to be uniformly modified, and in fact more than one of the aforementioned modifications can be incorporated in a single compound or even at a single nucleoside within an iRNA. The present invention also includes iRNA compounds that are chimeric compounds.

"Chimeric" iRNA compounds or "chimeras," in the context of this invention, are iRNA compounds, preferably dsRNAi agents, that contain two or more chemically distinct regions, each made up of at least one monomer unit, i.e., a nucleotide in the case of a dsRNA compound. These iRNAs typically contain at least one region wherein the RNA is modified so as to confer upon the iRNA increased resistance to nuclease degradation, increased cellular uptake, or increased binding affinity for the target nucleic acid. An additional region of the iRNA can serve as a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. By way of example, RNase H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNA duplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of iRNA inhibition of gene expression. Consequently, comparable results can often be obtained with shorter iRNAs when chimeric dsRNAs are used, compared to phosphorothioate deoxy dsRNAs hybridizing to the same target region. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art.

In certain instances, the RNA of an iRNA can be modified by a non-ligand group. A number of non-ligand molecules have been conjugated to iRNAs in order to enhance the activity, cellular distribution or cellular uptake of the iRNA, and procedures for performing such conjugations are available in the scientific literature. Such non-ligand moieties have included lipid moieties, such as cholesterol (Kubo, T. et al., *Biochem. Biophys. Res. Comm.,* 2007, 365(1):54-61; Letsinger et al., *Proc. Natl. Acad. Sci. USA,* 1989, 86:6553), cholic acid (Manoharan et al., *Bioorg. Med. Chem. Lett.,* 1994, 4:1053), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., *Ann. N.Y. Acad. Sci.,* 1992, 660:306; Manoharan et al., *Bioorg. Med. Chem. Let.,* 1993, 3:2765), a thiocholesterol (Oberhauser et al., *Nucl. Acids Res.,* 1992, 20:533), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., *EMBO J.,* 1991, 10:111; Kabanov et al., *FEBS Lett.,* 1990, 259:327; Svinarchuk et al., *Biochimie,* 1993, 75:49), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., *Tetrahedron Lett.,* 1995, 36:3651; Shea et al., *Nucl. Acids Res.,* 1990, 18:3777), a polyamine or a polyethylene glycol chain (Manoharan et al., *Nucleosides & Nucleotides,* 1995, 14:969), or adamantane acetic acid (Manoharan et al., *Tetrahedron Lett.,* 1995, 36:3651), a palmityl moiety (Mishra et al., *Biochim. Biophys. Acta,* 1995, 1264:229), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., *J. Pharmacol. Exp. Ther.,* 1996, 277:923). Representative United States patents that teach the preparation of such RNA conjugates have been listed above. Typical conjugation protocols involve the synthesis of RNAs bearing an aminolinker at one or more positions of the sequence. The amino group is then reacted with the molecule being conjugated using appropriate coupling or activating reagents. The conjugation reaction can be performed either with the RNA still bound to the solid support or following cleavage of the RNA, in solution phase. Purification of the RNA conjugate by HPLC typically affords the pure conjugate.

IV. Delivery of an iRNA of the Invention

The delivery of an iRNA of the invention to a cell e.g., a cell within a subject, such as a human subject (e.g., a subject in need thereof, such as a subject having a disease, disorder, or condition associated with XDH gene expression) can be achieved in a number of different ways. For example, delivery may be performed by contacting a cell with an iRNA of the invention either in vitro or in vivo. In vivo delivery may also be performed directly by administering a composition comprising an iRNA, e.g., a dsRNA, to a subject. Alternatively, in vivo delivery may be performed indirectly by administering one or more vectors that encode and direct the expression of the iRNA. These alternatives are discussed further below.

In general, any method of delivering a nucleic acid molecule (in vitro or in vivo) can be adapted for use with an iRNA of the invention (see e.g., Akhtar S and Julian R L. (1992) *Trends Cell. Biol.* 2(5):139-144 and WO94/02595, which are incorporated herein by reference in their entireties). For in vivo delivery, factors to consider in order to deliver an iRNA molecule include, for example, biological stability of the delivered molecule, prevention of non-specific effects, and accumulation of the delivered molecule in the target tissue. The non-specific effects of an iRNA can be minimized by local administration, for example, by direct injection or implantation into a tissue or topically administering the preparation. Local administration to a treatment site maximizes local concentration of the agent, limits the exposure of the agent to systemic tissues that can otherwise be harmed by the agent or that can degrade the agent, and permits a lower total dose of the iRNA molecule to be administered. Several studies have shown successful knockdown of gene products when a dsRNAi agent is administered locally. For example, intraocular delivery of a VEGF dsRNA by intravitreal injection in cynomolgus monkeys (Tolentino, M J, et al (2004) *Retina* 24:132-138) and subretinal injections in mice (Reich, S J., et al (2003) *Mol. Vis.* 9:210-216) were both shown to prevent neovascularization in an experimental model of age-related macular degeneration. In addition, direct intratumoral injection of a dsRNA in mice reduces tumor volume (Pille, J., et al (2005) *Mol. Ther.* 11:267-274) and can prolong survival of tumor-bearing mice (Kim, W J., et al (2006) *Mol. Ther.* 14:343-350; Li, S., et al (2007) *Mol. Ther.* 15:515-523). RNA interference has also shown success with local delivery to the CNS by direct injection (Dorn, G., et al. (2004) *Nucleic Acids* 32:e49; Tan, P H., et al (2005) *Gene Ther.* 12:59-66; Makimura, H., et al (2002) *BMC Neurosci.* 3:18; Shishkina, G T., et al (2004) *Neuroscience* 129:521-528; Thakker, E R., et al (2004) *Proc. Natl. Acad. Sci. U.S.A.* 101:17270-17275; Akaneya,Y., et al (2005) *J. Neurophysiol.* 93:594-602) and to the lungs by

intranasal administration (Howard, K A., et al (2006) *Mol. Ther.* 14:476-484; Zhang, X., et al (2004) *J. Biol. Chem.* 279:10677-10684; Bitko, V., et al (2005) *Nat. Med.* 11:50-55). For administering an iRNA systemically for the treatment of a disease, the RNA can be modified or alternatively delivered using a drug delivery system; both methods act to prevent the rapid degradation of the dsRNA by endo- and exo-nucleases in vivo. Modification of the RNA or the pharmaceutical carrier can also permit targeting of the iRNA to the target tissue and avoid undesirable off-target effects. iRNA molecules can be modified by chemical conjugation to lipophilic groups such as cholesterol to enhance cellular uptake and prevent degradation. For example, an iRNA directed against ApoB conjugated to a lipophilic cholesterol moiety was injected systemically into mice and resulted in knockdown of apoB mRNA in both the liver and jejunum (Soutschek, J., et al (2004) *Nature* 432:173-178). Conjugation of an iRNA to an aptamer has been shown to inhibit tumor growth and mediate tumor regression in a mouse model of prostate cancer (McNamara, J O, et al (2006) *Nat. Biotechnol.* 24:1005-1015). In an alternative embodiment, the iRNA can be delivered using drug delivery systems such as a nanoparticle, a dendrimer, a polymer, liposomes, or a cationic delivery system. Positively charged cationic delivery systems facilitate binding of an iRNA molecule (negatively charged) and also enhance interactions at the negatively charged cell membrane to permit efficient uptake of an iRNA by the cell. Cationic lipids, dendrimers, or polymers can either be bound to an iRNA, or induced to form a vesicle or micelle (see e.g., Kim S H, et al (2008) *Journal of Controlled Release* 129(2):107-116) that encases an iRNA. The formation of vesicles or micelles further prevents degradation of the iRNA when administered systemically. Methods for making and administering cationic-iRNA complexes are well within the abilities of one skilled in the art (see e.g., Sorensen, D R, et al (2003) *J. Mol. Biol* 327:761-766; Verma, U N, et al (2003) *Clin. Cancer Res.* 9:1291-1300; Arnold, A S et al (2007) *J. Hypertens.* 25:197-205, which are incorporated herein by reference in their entirety). Some non-limiting examples of drug delivery systems useful for systemic delivery of iRNAs include DOTAP (Sorensen, D R., et al (2003), supra; Verma, U N, et al (2003), supra), Oligofectamine, "solid nucleic acid lipid particles" (Zimmermann, T S, et al (2006) *Nature* 441:111-114), cardiolipin (Chien, P Y, et al (2005) *Cancer Gene Ther.* 12:321-328; Pal, A, et al (2005) *Int J. Oncol.* 26:1087-1091), polyethyleneimine (Bonnet M E, et al (2008) *Pharm. Res.* August 16 Epub ahead of print; Aigner, A. (2006) *J. Biomed. Biotechnol.* 71659), Arg-Gly-Asp (RGD) peptides (Liu, S. (2006) *Mol. Pharm.* 3:472-487), and polyamidoamines (Tomalia, D A, et al (2007) *Biochem. Soc. Trans.* 35:61-67; Yoo, H., et al (1999) *Pharm. Res.* 16:1799-1804). In some embodiments, an iRNA forms a complex with cyclodextrin for systemic administration. Methods for administration and pharmaceutical compositions of iRNAs and cyclodextrins can be found in U.S. Pat. No. 7,427,605, which is herein incorporated by reference in its entirety.

A. Vector Encoded iRNAs of the Invention

iRNA targeting the XDH gene can be expressed from transcription units inserted into DNA or RNA vectors (see, e.g., Couture, A, et al., *TIG.* (1996), 12:5-10; Skillern, A, et al., International PCT Publication No. WO 00/22113, Conrad, International PCT Publication No. WO 00/22114, and Conrad, U.S. Pat. No. 6,054,299). Expression can be transient (on the order of hours to weeks) or sustained (weeks to months or longer), depending upon the specific construct used and the target tissue or cell type. These transgenes can be introduced as a linear construct, a circular plasmid, or a viral vector, which can be an integrating or non-integrating vector. The transgene can also be constructed to permit it to be inherited as an extrachromosomal plasmid (Gassmann, et al., *Proc. Natl. Acad. Sci. USA* (1995) 92:1292).

The individual strand or strands of an iRNA can be transcribed from a promoter on an expression vector. Where two separate strands are to be expressed to generate, for example, a dsRNA, two separate expression vectors can be co-introduced (e.g., by transfection or infection) into a target cell. Alternatively each individual strand of a dsRNA can be transcribed by promoters both of which are located on the same expression plasmid. In one embodiment, a dsRNA is expressed as inverted repeat polynucleotides joined by a linker polynucleotide sequence such that the dsRNA has a stem and loop structure.

iRNA expression vectors are generally DNA plasmids or viral vectors. Expression vectors compatible with eukaryotic cells, preferably those compatible with vertebrate cells, can be used to produce recombinant constructs for the expression of an iRNA as described herein. Eukaryotic cell expression vectors are well known in the art and are available from a number of commercial sources. Typically, such vectors are provided containing convenient restriction sites for insertion of the desired nucleic acid segment. Delivery of iRNA expressing vectors can be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that allows for introduction into a desired target cell.

Viral vector systems which can be utilized with the methods and compositions described herein include, but are not limited to, (a) adenovirus vectors; (b) retrovirus vectors, including but not limited to lentiviral vectors, moloney murine leukemia virus, etc.; (c) adeno-associated virus vectors; (d) herpes simplex virus vectors; (e) SV 40 vectors; (f) polyoma virus vectors; (g) papilloma virus vectors; (h) picornavirus vectors; (i) pox virus vectors such as an orthopox, e.g., vaccinia virus vectors or avipox, e.g. canary pox or fowl pox; and (j) a helper-dependent or gutless adenovirus. Replication-defective viruses can also be advantageous. Different vectors will or will not become incorporated into the cells' genome. The constructs can include viral sequences for transfection, if desired. Alternatively, the construct can be incorporated into vectors capable of episomal replication, e.g. EPV and EBV vectors. Constructs for the recombinant expression of an iRNA will generally require regulatory elements, e.g., promoters, enhancers, etc., to ensure the expression of the iRNA in target cells. Other aspects to consider for vectors and constructs are known in the art.

V. Pharmaceutical Compositions of the Invention

The present invention also includes pharmaceutical compositions and formulations which include the iRNAs of the invention. In one embodiment, provided herein are pharmaceutical compositions containing an iRNA, as described herein, and a pharmaceutically acceptable carrier. The pharmaceutical compositions containing the iRNA are useful for treating a disease or disorder associated with the expression or activity of an XDH gene. Such pharmaceutical compositions are formulated based on the mode of delivery. One example is compositions that are formulated for systemic administration via parenteral delivery, e.g., by subcutaneous (SC), intramuscular (IM), or intravenous (IV) delivery. The

pharmaceutical compositions of the invention may be administered in dosages sufficient to inhibit expression of an XDH gene.

The pharmaceutical compositions of the invention may be administered in dosages sufficient to inhibit expression of an XDH gene. In general, a suitable dose of an iRNA of the invention will be in the range of about 0.001 to about 200.0 milligrams per kilogram body weight of the recipient per day, generally in the range of about 1 to 50 mg per kilogram body weight per day. Typically, a suitable dose of an iRNA of the invention will be in the range of about 0.1 mg/kg to about 5.0 mg/kg, preferably about 0.3 mg/kg and about 3.0 mg/kg. A repeat-dose regimine may include administration of a therapeutic amount of iRNA on a regular basis, such as every other day or once a year. In certain embodiments, the iRNA is administered about once per month to about once per quarter (i.e., about once every three months).

After an initial treatment regimen, the treatments can be administered on a less frequent basis. For example, after administration weekly or biweekly for three months, administration can be repeated once per month, for six months, or a year; or longer.

The pharmaceutical composition can be administered once daily, or the iRNA can be administered as two, three, or more sub-doses at appropriate intervals throughout the day or even using continuous infusion or delivery through a controlled release formulation. In that case, the iRNA contained in each sub-dose must be correspondingly smaller in order to achieve the total daily dosage. The dosage unit can also be compounded for delivery over several days, e.g., using a conventional sustained release formulation which provides sustained release of the iRNA over a several day period. Sustained release formulations are well known in the art and are particularly useful for delivery of agents at a particular site, such as could be used with the agents of the present invention. In this embodiment, the dosage unit contains a corresponding multiple of the daily dose.

In other embodiments, a single dose of the pharmaceutical compositions can be long lasting, such that subsequent doses are administered at not more than 3, 4, or 5 day intervals, or at not more than 1, 2, 3, or 4 week intervals. In some embodiments of the invention, a single dose of the pharmaceutical compositions of the invention is administered once per week. In other embodiments of the invention, a single dose of the pharmaceutical compositions of the invention is administered bi-monthly.

The skilled artisan will appreciate that certain factors can influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a composition can include a single treatment or a series of treatments. Estimates of effective dosages and in vivo half-lives for the individual iRNAs encompassed by the invention can be made using conventional methodologies or on the basis of in vivo testing using an appropriate animal model, as known in the art. For example, both genetic and induced models of hyperuricemia are known in the art. Genetic models of hyperuricemia include the B6; 129S7-Uox^{tm1Bay}/J mouse available from Jackson Laboratory (/jaxmice.jax.org/strain/002223.html) which develops hyperuricemia, with 10-fold higher levels of serum uric acid levels. Alternatively, hyperuricemia can be included in rats by feeing with a uricase inhibitor (oxonic acid) in the diet (Mazzali et al., Hypertension 38:1101-1106, 2001; Habu et al., Biochem. Pharmacol. 66:1107-1114, 2003). Unlike humans, rats and mice have uricase which metabolises uric acid, therefore, the enzyme must be inhibited in small animal models. Such models and considerations are well known in the art.

The pharmaceutical compositions of the present invention can be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration can be topical (e.g., by a transdermal patch), pulmonary, e.g., by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal, oral or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal, or intramuscular injection or infusion; subdermal, e.g., via an implanted device; or intracranial, e.g., by intraparenchymal, intrathecal or intraventricular administration.

The iRNA can be delivered in a manner to target a particular tissue (e.g., vascular endothelial cells).

Pharmaceutical compositions and formulations for topical or transdermal administration can include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like can be necessary or desirable. Coated condoms, gloves and the like can also be useful. Suitable topical formulations include those in which the iRNAs featured in the invention are in admixture with a topical delivery agent such as lipids, liposomes, fatty acids, fatty acid esters, steroids, chelating agents and surfactants. Suitable lipids and liposomes include neutral (e.g., dioleoylphosphatidyl DOPE ethanolamine, dimyristoylphosphatidyl choline DMPC, distearolyphosphatidyl choline) negative (e.g., dimyristoylphosphatidyl glycerol DMPG) and cationic (e.g., dioleoyltetramethylaminopropyl DOTAP and dioleoylphosphatidyl ethanolamine DOTMA). iRNAs featured in the invention can be encapsulated within liposomes or can form complexes thereto, in particular to cationic liposomes. Alternatively, iRNAs can be complexed to lipids, in particular to cationic lipids. Suitable fatty acids and esters include but are not limited to arachidonic acid, oleic acid, eicosanoic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a C_{1-20} alkyl ester (e.g., isopropylmyristate IPM), monoglyceride, diglyceride or pharmaceutically acceptable salt thereof). Topical formulations are described in detail in U.S. Pat. No. 6,747,014, which is incorporated herein by reference.

A. iRNA Formulations Comprising Membranous Molecular Assemblies

An iRNA for use in the compositions and methods of the invention can be formulated for delivery in a membranous molecular assembly, e.g., a liposome or a micelle. As used herein, the term "liposome" refers to a vesicle composed of amphiphilic lipids arranged in at least one bilayer, e.g., one bilayer or a plurality of bilayers. Liposomes include unilamellar and multilamellar vesicles that have a membrane formed from a lipophilic material and an aqueous interior. The aqueous portion contains the iRNA. The lipophilic material isolates the aqueous interior from an aqueous exterior, which typically does not include the iRNA composition, although in some examples, it may. Liposomes are useful for the transfer and delivery of active ingredients to the site of action. Because the liposomal membrane is structurally similar to biological membranes, when liposomes are applied to a tissue, the liposomal bilayer fuses

with bilayer of the cellular membranes. As the merging of the liposome and cell progresses, the internal aqueous contents that include the iRNA are delivered into the cell where the iRNA can specifically bind to a target RNA and can mediate RNA interference. In some cases the liposomes are also specifically targeted, e.g., to direct the iRNA to particular cell types.

A liposome containing an iRNA agent can be prepared by a variety of methods. In one example, the lipid component of a liposome is dissolved in a detergent so that micelles are formed with the lipid component. For example, the lipid component can be an amphipathic cationic lipid or lipid conjugate. The detergent can have a high critical micelle concentration and may be nonionic. Exemplary detergents include cholate, CHAPS, octylglucoside, deoxycholate, and lauroyl sarcosine. The iRNA agent preparation is then added to the micelles that include the lipid component. The cationic groups on the lipid interact with the iRNA agent and condense around the iRNA agent to form a liposome. After condensation, the detergent is removed, e.g., by dialysis, to yield a liposomal preparation of iRNA agent.

If necessary a carrier compound that assists in condensation can be added during the condensation reaction, e.g., by controlled addition. For example, the carrier compound can be a polymer other than a nucleic acid (e.g., spermine or spermidine). pH can also adjusted to favor condensation.

Methods for producing stable polynucleotide delivery vehicles, which incorporate a polynucleotide/cationic lipid complex as structural components of the delivery vehicle, are further described in, e.g., WO 96/37194, the entire contents of which are incorporated herein by reference. Liposome formation can also include one or more aspects of exemplary methods described in Feigner, P. L. et al., *Proc. Natl. Acad. Sci., USA* 8:7413-7417, 1987; U.S. Pat. Nos. 4,897,355; 5,171,678; Bangham, et al. *M. Mol. Biol.* 23:238, 1965; Olson, et al. *Biochim. Biophys. Acta* 557:9, 1979; Szoka, et al. *Proc. Natl. Acad. Sci.* 75: 4194, 1978; Mayhew, et al. *Biochim. Biophys. Acta* 775:169, 1984; Kim, et al. *Biochim. Biophys. Acta* 728:339, 1983; and Fukunaga, et al. *Endocrinol.* 115:757, 1984. Commonly used techniques for preparing lipid aggregates of appropriate size for use as delivery vehicles include sonication and freeze-thaw plus extrusion (see, e.g., Mayer, et al. *Biochim. Biophys. Acta* 858:161, 1986). Microfluidization can be used when consistently small (50 to 200 nm) and relatively uniform aggregates are desired (Mayhew, et al. *Biochim. Biophys. Acta* 775:169, 1984). These methods are readily adapted to packaging iRNA agent preparations into liposomes.

Liposomes fall into two broad classes. Cationic liposomes are positively charged liposomes which interact with the negatively charged nucleic acid molecules to form a stable complex. The positively charged nucleic acid/liposome complex binds to the negatively charged cell surface and is internalized in an endosome. Due to the acidic pH within the endosome, the liposomes are ruptured, releasing their contents into the cell cytoplasm (Wang et al., *Biochem. Biophys. Res. Commun.,* 1987, 147, 980-985).

Liposomes which are pH-sensitive or negatively-charged, entrap nucleic acids rather than complex with it. Since both the nucleic acid and the lipid are similarly charged, repulsion rather than complex formation occurs. Nevertheless, some nucleic acid is entrapped within the aqueous interior of these liposomes. pH-sensitive liposomes have been used to deliver nucleic acids encoding the thymidine kinase gene to cell monolayers in culture. Expression of the exogenous gene was detected in the target cells (Zhou et al., *Journal of Controlled Release,* 1992, 19, 269-274).

One major type of liposomal composition includes phospholipids other than naturally-derived phosphatidylcholine. Neutral liposome compositions, for example, can be formed from dimyristoyl phosphatidylcholine (DMPC) or dipalmitoyl phosphatidylcholine (DPPC). Anionic liposome compositions generally are formed from dimyristoyl phosphatidylglycerol, while anionic fusogenic liposomes are formed primarily from dioleoyl phosphatidylethanolamine (DOPE). Another type of liposomal composition is formed from phosphatidylcholine (PC) such as, for example, soybean PC, and egg PC. Another type is formed from mixtures of two or more of phospholipid, phosphatidylcholine, and cholesterol.

Examples of other methods to introduce liposomes into cells in vitro and in vivo include U.S. Pat. Nos. 5,283,185 and 5,171,678; WO 94/00569; WO 93/24640; WO 91/16024; Feigner, *J. Biol. Chem.* 269:2550, 1994; Nabel, *Proc. Natl. Acad. Sci.* 90:11307, 1993; Nabel, *Human Gene Ther.* 3:649, 1992; Gershon, *Biochem.* 32:7143, 1993; and Strauss *EMBO J.* 11:417, 1992.

Non-ionic liposomal systems have also been examined to determine their utility in the delivery of drugs to the skin, in particular systems comprising non-ionic surfactant and cholesterol. Non-ionic liposomal formulations comprising Novasome™ I (glyceryl dilaurate/cholesterol/polyoxyethylene-10-stearyl ether) and Novasome™ II (glyceryl distearate/cholesterol/polyoxyethylene-10-stearyl ether) were used to deliver cyclosporin-A into the dermis of mouse skin. Results indicated that such non-ionic liposomal systems were effective in facilitating the deposition of cyclosporine A into different layers of the skin (Hu et al. *S.T.P. Pharma. Sci.,* 1994, 4(6) 466).

Liposomes also include "sterically stabilized" liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids that, when incorporated into liposomes, result in enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. Examples of sterically stabilized liposomes are those in which part of the vesicle-forming lipid portion of the liposome (A) comprises one or more glycolipids, such as monosialoganglioside G_{M1}, or (B) is derivatized with one or more hydrophilic polymers, such as a polyethylene glycol (PEG) moiety. While not wishing to be bound by any particular theory, it is thought in the art that, at least for sterically stabilized liposomes containing gangliosides, sphingomyelin, or PEG-derivatized lipids, the enhanced circulation half-life of these sterically stabilized liposomes derives from a reduced uptake into cells of the reticuloendothelial system (RES) (Allen et al., *FEBS Letters,* 1987, 223, 42; Wu et al., *Cancer Research,* 1993, 53, 3765).

Various liposomes comprising one or more glycolipids are known in the art. Papahadjopoulos et al. (*Ann. N.Y. Acad. Sci.,* 1987, 507, 64) reported the ability of monosialoganglioside G_{M1}, galactocerebroside sulfate and phosphatidylinositol to improve blood half-lives of liposomes. These findings were expounded upon by Gabizon et al. (*Proc. Natl. Acad. Sci. U.S.A.,* 1988, 85, 6949). U.S. Pat. No. 4,837,028 and WO 88/04924, both to Allen et al., disclose liposomes comprising (1) sphingomyelin and (2) the ganglioside G_{M1} or a galactocerebroside sulfate ester. U.S. Pat. No. 5,543,152 (Webb et al.) discloses liposomes comprising sphingomyelin. Liposomes comprising 1,2-sn-dimyristoylphosphatidylcholine are disclosed in WO 97/13499 (Lim et al).

In some embodiments, cationic liposomes are used. Cationic liposomes possess the advantage of being able to fuse to the cell membrane. Non-cationic liposomes, although not able to fuse as efficiently with the plasma membrane, are

taken up by macrophages in vivo and can be used to deliver iRNA agents to macrophages.

Further advantages of liposomes include: liposomes obtained from natural phospholipids are biocompatible and biodegradable; liposomes can incorporate a wide range of water and lipid soluble drugs; liposomes can protect encapsulated iRNAs in their internal compartments from metabolism and degradation (Rosoff, in "Pharmaceutical Dosage Forms," Lieberman, Rieger and Banker (Eds.), 1988, volume 1, p. 245). Important considerations in the preparation of liposome formulations are the lipid surface charge, vesicle size, and the aqueous volume of the liposomes.

A positively charged synthetic cationic lipid, N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA) can be used to form small liposomes that interact spontaneously with nucleic acid to form lipid-nucleic acid complexes which are capable of fusing with the negatively charged lipids of the cell membranes of tissue culture cells, resulting in delivery of iRNA agent (see, e.g., Feigner, P. L. et al., Proc. Natl. Acad. Sci., USA 8:7413-7417, 1987 and U.S. Pat. No. 4,897,355 for a description of DOTMA and its use with DNA).

A DOTMA analogue, 1,2-bis(oleoyloxy)-3-(trimethylammonia)propane (DOTAP) can be used in combination with a phospholipid to form DNA-complexing vesicles. Lipofectin™ (Bethesda Research Laboratories, Gaithersburg, Md.) is an effective agent for the delivery of highly anionic nucleic acids into living tissue culture cells that comprise positively charged DOTMA liposomes which interact spontaneously with negatively charged polynucleotides to form complexes. When enough positively charged liposomes are used, the net charge on the resulting complexes is also positive. Positively charged complexes prepared in this way spontaneously attach to negatively charged cell surfaces, fuse with the plasma membrane, and efficiently deliver functional nucleic acids into, for example, tissue culture cells. Another commercially available cationic lipid, 1,2-bis (oleoyloxy)-3,3-(trimethylammonia)propane ("DOTAP") (Boehringer Mannheim, Indianapolis, Ind.) differs from DOTMA in that the oleoyl moieties are linked by ester, rather than ether linkages.

Other reported cationic lipid compounds include those that have been conjugated to a variety of moieties including, for example, carboxyspermine which has been conjugated to one of two types of lipids and includes compounds such as 5-carboxyspermylglycine dioctaoleoylamide ("DOGS") (Transfectam™, Promega, Madison, Wis.) and dipalmitoylphosphatidylethanolamine 5-carboxyspermyl-amide ("DPPES") (see, e.g., U.S. Pat. No. 5,171,678).

Another cationic lipid conjugate includes derivatization of the lipid with cholesterol ("DC-Chol") which has been formulated into liposomes in combination with DOPE (See, Gao, X. and Huang, L., *Biochim. Biophys. Res. Commun.* 179:280, 1991). Lipopolylysine, made by conjugating polylysine to DOPE, has been reported to be effective for transfection in the presence of serum (Zhou, X. et al., *Biochim. Biophys. Acta* 1065:8, 1991). For certain cell lines, these liposomes containing conjugated cationic lipids, are said to exhibit lower toxicity and provide more efficient transfection than the DOTMA-containing compositions. Other commercially available cationic lipid products include DMRIE and DMRIE-HP (Vical, La Jolla, Calif.) and Lipofectamine (DOSPA) (Life Technology, Inc., Gaithersburg, Md.). Other cationic lipids suitable for the delivery of oligonucleotides are described in WO 98/39359 and WO 96/37194.

Liposomal formulations are particularly suited for topical administration, liposomes present several advantages over other formulations. Such advantages include reduced side effects related to high systemic absorption of the administered drug, increased accumulation of the administered drug at the desired target, and the ability to administer iRNA agent into the skin. In some implementations, liposomes are used for delivering iRNA agent to epidermal cells and also to enhance the penetration of iRNA agent into dermal tissues, e.g., into skin. For example, the liposomes can be applied topically. Topical delivery of drugs formulated as liposomes to the skin has been documented (see, e.g., Weiner et al., *Journal of Drug Targeting,* 1992, vol. 2, 405-410 and du Plessis et al., *Antiviral Research,* 18, 1992, 259-265; Mannino, R. J. and Fould-Fogerite, S., *Biotechniques* 6:682-690, 1988; Itani, T. et al. *Gene* 56:267-276. 1987; Nicolau, C. et al. *Meth. Enz.* 149:157-176, 1987; Straubinger, R. M. and Papahadjopoulos, D. *Meth. Enz.* 101:512-527, 1983; Wang, C. Y. and Huang, *L., Proc. Natl. Acad. Sci. USA* 84:7851-7855, 1987).

Non-ionic liposomal systems have also been examined to determine their utility in the delivery of drugs to the skin, in particular systems comprising non-ionic surfactant and cholesterol. Non-ionic liposomal formulations comprising Novasome™ I (glyceryl dilaurate/cholesterol/polyoxyethylene-10-stearyl ether) and Novasome™ II (glyceryl distearate/cholesterol/polyoxyethylene-10-stearyl ether) were used to deliver a drug into the dermis of mouse skin. Such formulations with iRNA agent are useful for treating a dermatological disorder.

Liposomes that include iRNA can be made highly deformable. Such deformability can enable the liposomes to penetrate through pore that are smaller than the average radius of the liposome. For example, transfersomes are a type of deformable liposomes. Transferosomes can be made by adding surface edge activators, usually surfactants, to a standard liposomal composition. Transfersomes that include iRNAs can be delivered, for example, subcutaneously by infection in order to deliver iRNAs to keratinocytes in the skin. In order to cross intact mammalian skin, lipid vesicles must pass through a series of fine pores, each with a diameter less than 50 nm, under the influence of a suitable transdermal gradient. In addition, due to the lipid properties, these transferosomes can be self-optimizing (adaptive to the shape of pores, e.g., in the skin), self-repairing, and can frequently reach their targets without fragmenting, and often self-loading.

Other formulations amenable to the present invention are described in WO/2008/042973. Transfersomes are yet another type of liposomes, and are highly deformable lipid aggregates which are attractive candidates for drug delivery vehicles. Transfersomes can be described as lipid droplets which are so highly deformable that they are easily able to penetrate through pores which are smaller than the droplet. Transfersomes are adaptable to the environment in which they are used, e.g., they are self-optimizing (adaptive to the shape of pores in the skin), self-repairing, frequently reach their targets without fragmenting, and often self-loading. To make transfersomes it is possible to add surface edge-activators, usually surfactants, to a standard liposomal composition. Transfersomes have been used to deliver serum albumin to the skin. The transfersome-mediated delivery of serum albumin has been shown to be as effective as subcutaneous injection of a solution containing serum albumin.

Surfactants find wide application in formulations such as emulsions (including microemulsions) and liposomes. The most common way of classifying and ranking the properties

of the many different types of surfactants, both natural and synthetic, is by the use of the hydrophile/lipophile balance (HLB). The nature of the hydrophilic group (also known as the "head") provides the most useful means for categorizing the different surfactants used in formulations (Rieger, in "Pharmaceutical Dosage Forms", Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).

If the surfactant molecule is not ionized, it is classified as a nonionic surfactant. Nonionic surfactants find wide application in pharmaceutical and cosmetic products and are usable over a wide range of pH values. In general their HLB values range from 2 to about 18 depending on their structure. Nonionic surfactants include nonionic esters such as ethylene glycol esters, propylene glycol esters, glyceryl esters, polyglyceryl esters, sorbitan esters, sucrose esters, and ethoxylated esters. Nonionic alkanolamides and ethers such as fatty alcohol ethoxylates, propoxylated alcohols, and ethoxylated/propoxylated block polymers are also included in this class. The polyoxyethylene surfactants are the most popular members of the nonionic surfactant class.

If the surfactant molecule carries a negative charge when it is dissolved or dispersed in water, the surfactant is classified as anionic. Anionic surfactants include carboxylates such as soaps, acyl lactylates, acyl amides of amino acids, esters of sulfuric acid such as alkyl sulfates and ethoxylated alkyl sulfates, sulfonates such as alkyl benzene sulfonates, acyl isethionates, acyl taurates and sulfosuccinates, and phosphates. The most important members of the anionic surfactant class are the alkyl sulfates and the soaps.

If the surfactant molecule carries a positive charge when it is dissolved or dispersed in water, the surfactant is classified as cationic. Cationic surfactants include quaternary ammonium salts and ethoxylated amines. The quaternary ammonium salts are the most used members of this class.

If the surfactant molecule has the ability to carry either a positive or negative charge, the surfactant is classified as amphoteric. Amphoteric surfactants include acrylic acid derivatives, substituted alkylamides, N-alkylbetaines and phosphatides.

The use of surfactants in drug products, formulations and in emulsions has been reviewed (Rieger, in "Pharmaceutical Dosage Forms", Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).

The iRNA for use in the methods of the invention can also be provided as micellar formulations. "Micelles" are defined herein as a particular type of molecular assembly in which amphipathic molecules are arranged in a spherical structure such that all the hydrophobic portions of the molecules are directed inward, leaving the hydrophilic portions in contact with the surrounding aqueous phase. The converse arrangement exists if the environment is hydrophobic.

A mixed micellar formulation suitable for delivery through transdermal membranes may be prepared by mixing an aqueous solution of iRNA, an alkali metal C_8 to C_{22} alkyl sulphate, and a micelle forming compounds. Exemplary micelle forming compounds include lecithin, hyaluronic acid, pharmaceutically acceptable salts of hyaluronic acid, glycolic acid, lactic acid, chamomile extract, cucumber extract, oleic acid, linoleic acid, linolenic acid, monoolein, monooleates, monolaurates, borage oil, evening of primrose oil, menthol, trihydroxy oxo cholanyl glycine and pharmaceutically acceptable salts thereof, glycerin, polyglycerin, lysine, polylysine, triolein, polyoxyethylene ethers and analogues thereof, polidocanol alkyl ethers and analogues thereof, chenodeoxycholate, deoxycholate, and mixtures thereof. The micelle forming compounds may be added at the same time or after addition of the alkali metal alkyl sulphate. Mixed micelles will form with substantially any kind of mixing of the ingredients but vigorous mixing in order to provide smaller size micelles.

In one method a first micellar composition is prepared which contains the RNAi and at least the alkali metal alkyl sulphate. The first micellar composition is then mixed with at least three micelle forming compounds to form a mixed micellar composition. In another method, the micellar composition is prepared by mixing the RNAi, the alkali metal alkyl sulphate and at least one of the micelle forming compounds, followed by addition of the remaining micelle forming compounds, with vigorous mixing.

Phenol or m-cresol may be added to the mixed micellar composition to stabilize the formulation and protect against bacterial growth. Alternatively, phenol or m-cresol may be added with the micelle forming ingredients. An isotonic agent such as glycerin may also be added after formation of the mixed micellar composition.

For delivery of the micellar formulation as a spray, the formulation can be put into an aerosol dispenser and the dispenser is charged with a propellant. The propellant, which is under pressure, is in liquid form in the dispenser. The ratios of the ingredients are adjusted so that the aqueous and propellant phases become one, i.e., there is one phase. If there are two phases, it is necessary to shake the dispenser prior to dispensing a portion of the contents, e.g., through a metered valve. The dispensed dose of pharmaceutical agent is propelled from the metered valve in a fine spray.

Propellants may include hydrogen-containing chlorofluorocarbons, hydrogen-containing fluorocarbons, dimethyl ether and diethyl ether. In certain embodiments, HFA 134a (1,1,1,2 tetrafluoroethane) may be used.

The specific concentrations of the essential ingredients can be determined by relatively straightforward experimentation. For absorption through the oral cavities, it is often desirable to increase, e.g., at least double or triple, the dosage for through injection or administration through the gastrointestinal tract.

B. Lipid Particles

iRNAs, e.g., dsRNAi agents of in the invention may be fully encapsulated in a lipid formulation, e.g., a LNP, or other nucleic acid-lipid particle.

As used herein, the term "LNP" refers to a stable nucleic acid-lipid particle. LNPs typically contain a cationic lipid, a non-cationic lipid, and a lipid that prevents aggregation of the particle (e.g., a PEG-lipid conjugate). LNPs are extremely useful for systemic applications, as they exhibit extended circulation lifetimes following intravenous (i.v.) injection and accumulate at distal sites (e.g., sites physically separated from the administration site). LNPs include "pSPLP," which include an encapsulated condensing agent-nucleic acid complex as set forth in PCT Publication No. WO 00/03683. The particles of the present invention typically have a mean diameter of about 50 nm to about 150 nm, more typically about 60 nm to about 130 nm, more typically about 70 nm to about 110 nm, most typically about 70 nm to about 90 nm, and are substantially nontoxic. In addition, the nucleic acids when present in the nucleic acid-lipid particles of the present invention are resistant in aqueous solution to degradation with a nuclease. Nucleic acid-lipid particles and their method of preparation are disclosed in, e.g., U.S. Pat. Nos. 5,976,567; 5,981,501; 6,534,484; 6,586,410; 6,815,432; US Publication No. 2010/0324120 and PCT Publication No. WO 96/40964.

In one embodiment, the lipid to drug ratio (mass/mass ratio) (e.g., lipid to dsRNA ratio) will be in the range of from about 1:1 to about 50:1, from about 1:1 to about 25:1, from about 3:1 to about 15:1, from about 4:1 to about 10:1, from about 5:1 to about 9:1, or about 6:1 to about 9:1. Ranges intermediate to the above recited ranges are also contemplated to be part of the invention.

The cationic lipid can be, for example, N,N-dioleyl-N,N-dimethylammonium chloride (DODAC), N,N-distearyl-N,N-dimethylammonium bromide (DDAB), N—(I-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP), N—(I-(2,3-dioleyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA), N,N-dimethyl-2,3-dioleyloxy)propylamine (DODMA), 1,2-DiLinoleyloxy-N,N-dimethylaminopropane (DLinDMA), 1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLenDMA), 1,2-Dilinoleylcarbamoyloxy-3-dimethylaminopropane (DLin-C-DAP), 1,2-Dilinoleyoxy-3-(dimethylamino) acetoxypropane (DLin-DAC), 1,2-Dilinoleyoxy-3-morpholinopropane (DLin-MA), 1,2-Dilinoleoyl-3-dimethylaminopropane (DLinDAP), 1,2-Dilinoleylthio-3-dimethylaminopropane (DLin-S-DMA), 1-Linoleoyl-2-linoleyloxy-3-dimethylaminopropane (DLin-2-DMAP), 1,2-Dilinoleyloxy-3-trimethylaminopropane chloride salt (DLin-TMA.C1), 1,2-Dilinoleoyl-3-trimethylaminopropane chloride salt (DLin-TAP.C1), 1,2-Dilinoleyloxy-3-(N-methylpiperazino)propane (DLin-MPZ), or 3-(N,N-Dilinoleylamino)-1,2-propanediol (DLinAP), 3-(N,N-Dioleylamino)-1,2-propanedio (DOAP), 1,2-Dilinoleyloxo-3-(2-N,N-dimethylamino)ethoxypropane (DLin-EG-DMA), 1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA), 2,2-Dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA) or analogs thereof, (3aR,5s,6aS)-N,N-dimethyl-2,2-di((9Z,12Z)-octadeca-9,12-dienyl)tetrahydro-3aH-cyclopenta[d][1,3]dioxol-5-amine (ALN100), (6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-yl 4-(dimethylamino)butanoate (MC3), 1,1'-(2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl)(2-hydroxydodecyl)amino) ethyl)piperazin-1-yl)ethylazanediyl)didodecan-2-ol (Tech G1), or a mixture thereof. The cationic lipid can comprise from about 20 mol % to about 50 mol % or about 40 mol % of the total lipid present in the particle.

In some embodiments, the compound 2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane can be used to prepare lipid-siRNA nanoparticles.

In some embodiments, the lipid-siRNA particle includes 40% 2, 2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane: 10% DSPC: 40% Cholesterol: 10% PEG-C-DOMG (mole percent) with a particle size of 63.0±20 nm and a 0.027 siRNA/Lipid Ratio.

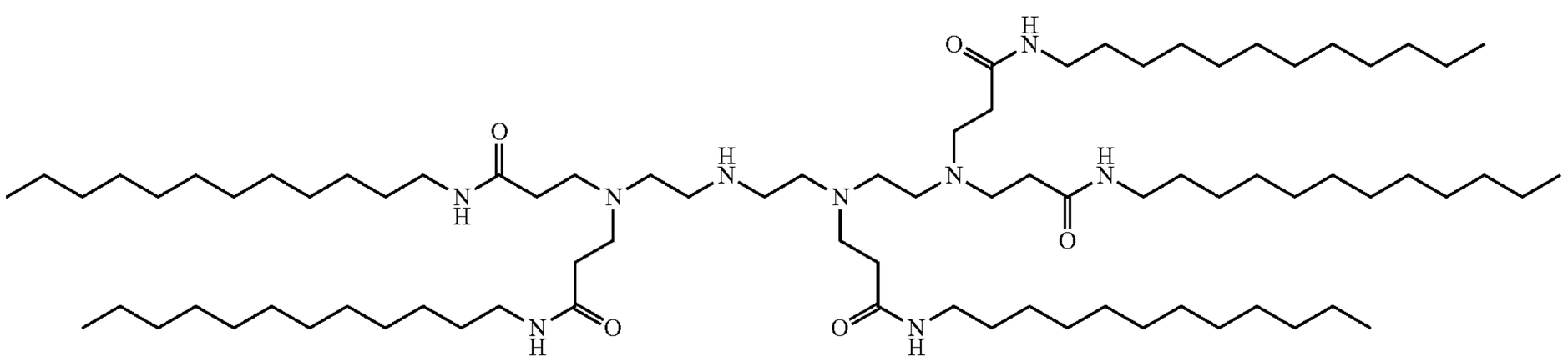
The ionizable/non-cationic lipid can be an anionic lipid or a neutral lipid including, but not limited to, distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoyl-phosphatidylethanolamine (DOPE), palmitoyloleoylphosphatidylcholine (POPC), palmitoyloleoylphosphatidylethanolamine (POPE), dioleoyl-phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidyl-ethanolamine (DSPE), 16-O-monomethyl PE, 16-O-dimethyl PE, 18-1-trans PE, 1-stearoyl-2-oleoyl-phosphatidyethanolamine (SOPE), cholesterol, or a mixture thereof. The non-cationic lipid can be from about 5 mol % to about 90 mol %, about 10 mol %, or about 58 mol % if cholesterol is included, of the total lipid present in the particle.

The conjugated lipid that inhibits aggregation of particles can be, for example, a polyethyleneglycol (PEG)-lipid including, without limitation, a PEG-diacylglycerol (DAG), a PEG-dialkyloxypropyl (DAA), a PEG-phospholipid, a PEG-ceramide (Cer), or a mixture thereof. The PEG-DAA conjugate can be, for example, a PEG-dilauryloxypropyl (Ci_2), a PEG-dimyristyloxypropyl (Ci_4), a PEG-dipalmityloxypropyl (Ci_6), or a PEG-distearyloxypropyl ($C]_8$). The conjugated lipid that prevents aggregation of particles can be from 0 mol % to about 20 mol % or about 2 mol % of the total lipid present in the particle.

In some embodiments, the nucleic acid-lipid particle further includes cholesterol at, e.g., about 10 mol % to about 60 mol % or about 48 mol % of the total lipid present in the particle.

In one embodiment, the lipidoid ND98.4HCl (MW 1487) (see U.S. patent application Ser. No. 12/056,230, filed Mar. 26, 2008, which is incorporated herein by reference), Cholesterol (Sigma-Aldrich), and PEG-Ceramide C16 (Avanti Polar Lipids) can be used to prepare lipid-dsRNA nanoparticles (i.e., LNP01 particles). Stock solutions of each in ethanol can be prepared as follows: ND98, 133 mg/ml; Cholesterol, 25 mg/ml, PEG-Ceramide C16, 100 mg/ml. The ND98, Cholesterol, and PEG-Ceramide C16 stock solutions can then be combined in a, e.g., 42:48:10 molar ratio. The combined lipid solution can be mixed with aqueous dsRNA (e.g., in sodium acetate pH 5) such that the final ethanol concentration is about 35-45% and the final sodium acetate concentration is about 100-300 mM. Lipid-dsRNA nanoparticles typically form spontaneously upon mixing. Depending on the desired particle size distribution, the resultant nanoparticle mixture can be extruded through a polycarbonate membrane (e.g., 100 nm cut-off) using, for example, a thermobarrel extruder, such as Lipex Extruder (Northern Lipids, Inc). In some cases, the extrusion step can be omitted. Ethanol removal and simultaneous buffer exchange can be accomplished by, for example, dialysis or tangential flow filtration. Buffer can be exchanged with, for example, phosphate buffered saline (PBS) at about pH 7, e.g., about pH 6.9, about pH 7.0, about pH 7.1, about pH 7.2, about pH 7.3, or about pH 7.4.

Formula 1



ND98 Isomer I

LNP01 formulations are described, e.g., in International Application Publication No. WO 2008/042973, which is hereby incorporated by reference.

Additional exemplary lipid-dsRNA formulations are described in Table 1.

TABLE 1

	Ionizable/Cationic Lipid	cationic lipid/non-cationic lipid/cholesterol/PEG-lipid conjugate Lipid:siRNA ratio
SNALP-1	1',2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA)	DLinDMA/DPPC/Cholesterol/PEG-cDMA (57.1/7.1/34.4/1.4) lipid:siRNA~7:1
2-XTC	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DPPC/Cholesterol/PEG-cDMA 57.1/7.1/34.4/1.4 lipid:siRNA~7:1
LNP05	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 57.5/7.5/31.5/3.5 lipid:siRNA~6:1
LNP06	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 57.5/7.5/31.5/3.5 lipid:siRNA~11:1
LNP07	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 60/7.5/31/1.5, lipid:siRNA~6:1
LNP08	2,2-Dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 60/7.5/31/1.5, lipid:siRNA~11:1
LNP09	2,2-Dilinoleyl-4-dimethylaminoethy1-[1,3]-dioxolane (XTC)	XTC/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP10	(3aR,5s,6aS)-N,N-dimethy1-2,2-di((9Z,12Z)-octadeca-9,12-dienyl)tetrahydro-3aH-cyclopenta[d][1,3]dioxol-5-amine (ALN100)	ALN100/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP11	(6Z,9Z,28Z,31Z)-heptatriaconta-6,9,28,31-tetraen-19-y1 4-(dimethylamino)butanoate (MC3)	MC-3/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP12	1,1'-(2-(4-(2-((2-(bis(2-hydroxydodecyl)amino)ethyl)(2-hydroxydodecyl)amino)ethyl)piperazin-1-yl)ethylazanediyl)didodecan-2-ol (Tech G1)	Tech Gl/DSPC/Cholesterol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA 10:1
LNP13	XTC	XTC/DSPC/Chol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA: 33:1
LNP14	MC3	MC3/DSPC/Chol/PEG-DMG 40/15/40/5 Lipid:siRNA: 11:1
LNP15	MC3	MC3/DSPC/Chol/PEG-DSG/GalNAc-PEG-DSG 50/10/35/4.5/0.5 Lipid:siRNA: 11:1
LNP16	MC3	MC3/DSPC/Chol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA: 7:1
LNP17	MC3	MC3/DSPC/Chol/PEG-DSG 50/10/38.5/1.5 Lipid:siRNA: 10:1
LNP18	MC3	MC3/DSPC/Chol/PEG-DMG 50/10/38.5/1.5 Lipid:siRNA: 12:1
LNP19	MC3	MC3/DSPC/Chol/PEG-DMG 50/10/35/5 Lipid:siRNA: 8:1
LNP20	MC3	MC3/DSPC/Chol/PEG-DPG 50/10/38.5/1.5 Lipid:siRNA: 10:1
LNP21	C12-200	C12-200/DSPC/Chol/PEG-DSG 50/10/38.5/1.5 Lipid:siRNA: 7:1

TABLE 1-continued

	Ionizable/Cationic Lipid	cationic lipid/non-cationic lipid/cholesterol/PEG-lipid conjugate Lipid:siRNA ratio
LNP22	XTC	XTC/DSPC/Chol/PEG-DSG 50/10/38.5/1.5 Lipid:siRNA: 10:1

DSPC: distearoylphosphatidylcholine

DPPC: dipalmitoylphosphatidylcholine

PEG-DMG: PEG-didimyristoyl glycerol (C14-PEG, or PEG-C14) (PEG with avg mol wt of 2000)

PEG-DSG: PEG-distyryl glycerol (C18-PEG, or PEG-C18) (PEG with avg mol wt of 2000)

PEG-cDMA: PEG-carbamoyl-1,2-dimyristyloxypropylamine (PEG with avg mol wt of 2000)

SNALP (1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLinDMA)) comprising formulations are described in International Publication No. WO2009/127060, filed Apr. 15, 2009, which is hereby incorporated by reference.

XTC comprising formulations are described, e.g., in International Patent Application No. PCT/US2010/022614, filed on Jan. 29, 2010, the entire content of which are hereby incorporated by reference.

MC3 comprising formulations are described, e.g., in US Patent Publication No. 2010/0324120, filed on Jun. 10, 2010, the entire contents of which are hereby incorporated by reference.

ALNY-100 comprising formulations are described, e.g., International Patent Application Number PCT/US09/63933, filed on Nov. 10, 2009, the entire contents of which are hereby incorporated by reference.

C12-200 comprising formulations are described in PCT Publication No. WO 2010/129709, the entire contents of which are hereby incorporated by reference.

Compositions and formulations for oral administration include powders or granules, microparticulates, nanoparticulates, suspensions or solutions in water or non-aqueous media, capsules, gel capsules, sachets, tablets or minitablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids, or binders can be desirable. In some embodiments, oral formulations are those in which dsRNAs featured in the invention are administered in conjunction with one or more penetration enhancer surfactants and chelators. Suitable surfactants include fatty acids or esters or salts thereof, bile acids or salts thereof. Suitable bile acids/salts include chenodeoxycholic acid (CDCA) and ursodeoxychenodeoxycholic acid (UDCA), cholic acid, dehydrocholic acid, deoxycholic acid, glucholic acid, glycholic acid, glycodeoxycholic acid, taurocholic acid, taurodeoxycholic acid, sodium tauro-24,25-dihydro-fusidate and sodium glycodihydrofusidate. Suitable fatty acids include arachidonic acid, undecanoic acid, oleic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a monoglyceride, a diglyceride or a pharmaceutically acceptable salt thereof (e.g., sodium). In some embodiments, combinations of penetration enhancers are used, for example, fatty acids/salts in combination with bile acids/salts. One exemplary combination is the sodium salt of lauric acid, capric acid and UDCA. Further penetration enhancers include polyoxyethylene-9-lauryl ether, polyoxyethylene-20-cetyl ether. DsRNAs featured in the invention can be delivered orally, in granular form including sprayed dried particles, or complexed to form micro or nanoparticles. DsRNA complexing agents include poly-amino acids; polyimines; polyacrylates; polyalkylacrylates, polyoxethanes, polyalkylcyanoacrylates; cationized gelatins, albumins, starches, acrylates, polyethyleneglycols (PEG), and starches; polyalkylcyanoacrylates; DEAE-derivatized polyimines, pollulans, celluloses, and starches. Suitable complexing agents include chitosan, N-trimethylchitosan, poly-L-lysine, polyhistidine, polyornithine, polyspermines, protamine, polyvinylpyridine, polythiodiethylaminomethylethylene P(TDAE), polyaminostyrene (e.g., p-amino), poly(methylcyanoacrylate), poly(ethylcyanoacrylate), poly(butylcyanoacrylate), poly(isobutylcyanoacrylate), poly(isohexylcynaoacrylate), DEAE-methacrylate, DEAE-hexylacrylate, DEAE-acrylamide, DEAE-albumin and DEAE-dextran, polymethylacrylate, polyhexylacrylate, poly(D,L-lactic acid), poly(DL-lactic-co-glycolic acid (PLGA), alginate, and polyethyleneglycol (PEG). Oral formulations for dsRNAs and their preparation are described in detail in U.S. Pat. No. 6,887,906, US Publn. No. 20030027780, and U.S. Pat. No. 6,747,014, each of which is incorporated herein by reference.

Compositions and formulations for parenteral, intraparenchymal (into the brain), intrathecal, intraventricular or intrahepatic administration can include sterile aqueous solutions which can also contain buffers, diluents, and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds, and other pharmaceutically acceptable carriers or excipients.

Pharmaceutical compositions of the present invention include, but are not limited to, solutions, emulsions, and liposome-containing formulations. These compositions can be generated from a variety of components that include, but are not limited to, preformed liquids, self-emulsifying solids, and self-emulsifying semisolids. Formulations include those that target the liver when treating hepatic disorders such as hepatic carcinoma.

The pharmaceutical formulations of the present invention, which can conveniently be presented in unit dosage form, can be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

The compositions of the present invention can be formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, gel capsules, liquid syrups, soft gels, suppositories, and enemas. The compositions of the present invention can also be formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions can further contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol or dextran. The suspension can also contain stabilizers.

C. Additional Formulations

i. Emulsions

The iRNAs of the present invention can be prepared and formulated as emulsions. Emulsions are typically heterogeneous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1 μm in diameter (see e.g.,

Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, L V., Popovich N G., and Ansel H C., 2004, Lippincott Williams & Wilkins (8th ed.), New York, N.Y.; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., Volume 1, p. 245; Block in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 2, p. 335; Higuchi et al., in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., 1985, p. 301). Emulsions are often biphasic systems comprising two immiscible liquid phases intimately mixed and dispersed with each other. In general, emulsions can be of either the water-in-oil (w/o) or the oil-in-water (o/w) variety. When an aqueous phase is finely divided into and dispersed as minute droplets into a bulk oily phase, the resulting composition is called a water-in-oil (w/o) emulsion. Alternatively, when an oily phase is finely divided into and dispersed as minute droplets into a bulk aqueous phase, the resulting composition is called an oil-in-water (o/w) emulsion. Emulsions can contain additional components in addition to the dispersed phases, and the active drug which can be present as a solution either in the aqueous phase, oily phase or itself as a separate phase. Pharmaceutical excipients such as emulsifiers, stabilizers, dyes, and anti-oxidants can also be present in emulsions as needed. Pharmaceutical emulsions can also be multiple emulsions that are comprised of more than two phases such as, for example, in the case of oil-in-water-in-oil (o/w/o) and water-in-oil-in-water (w/o/w) emulsions. Such complex formulations often provide certain advantages that simple binary emulsions do not. Multiple emulsions in which individual oil droplets of an o/w emulsion enclose small water droplets constitute a w/o/w emulsion. Likewise a system of oil droplets enclosed in globules of water stabilized in an oily continuous phase provides an o/w/o emulsion.

Emulsions are characterized by little or no thermodynamic stability. Often, the dispersed or discontinuous phase of the emulsion is well dispersed into the external or continuous phase and maintained in this form through the means of emulsifiers or the viscosity of the formulation. Either of the phases of the emulsion can be a semisolid or a solid, as is the case of emulsion-style ointment bases and creams. Other means of stabilizing emulsions entail the use of emulsifiers that can be incorporated into either phase of the emulsion. Emulsifiers can broadly be classified into four categories: synthetic surfactants, naturally occurring emulsifiers, absorption bases, and finely dispersed solids (see e.g., Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, L V., Popovich N G., and Ansel H C., 2004, Lippincott Williams & Wilkins (8th ed.), New York, N.Y.; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

Synthetic surfactants, also known as surface active agents, have found wide applicability in the formulation of emulsions and have been reviewed in the literature (see e.g., Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, L V., Popovich N G., and Ansel H C., 2004, Lippincott Williams & Wilkins (8th ed.), New York, N.Y.; Rieger, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), Marcel Dekker, Inc., New York, N.Y., 1988, volume 1, p. 199). Surfactants are typically amphiphilic and comprise a hydrophilic and a hydrophobic portion. The ratio of the hydrophilic to the hydrophobic nature of the surfactant has been termed the hydrophile/lipophile balance (HLB) and is a valuable tool in categorizing and selecting surfactants in the preparation of formulations. Surfactants can be classified into different classes based on the nature of the hydrophilic group: nonionic, anionic, cationic, and amphoteric (see e.g., Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, L V., Popovich N G., and Ansel H C., 2004, Lippincott Williams & Wilkins (8th ed.), New York, N.Y. Rieger, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285).

Naturally occurring emulsifiers used in emulsion formulations include lanolin, beeswax, phosphatides, lecithin and acacia. Absorption bases possess hydrophilic properties such that they can soak up water to form w/o emulsions yet retain their semisolid consistencies, such as anhydrous lanolin and hydrophilic petrolatum. Finely divided solids have also been used as good emulsifiers especially in combination with surfactants and in viscous preparations. These include polar inorganic solids, such as heavy metal hydroxides, nonswelling clays such as bentonite, attapulgite, hectorite, kaolin, montmorillonite, colloidal aluminum silicate, and colloidal magnesium aluminum silicate, pigments and nonpolar solids such as carbon or glyceryl tristearate.

A large variety of non-emulsifying materials are also included in emulsion formulations and contribute to the properties of emulsions. These include fats, oils, waxes, fatty acids, fatty alcohols, fatty esters, humectants, hydrophilic colloids, preservatives, and antioxidants (Block, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

Hydrophilic colloids or hydrocolloids include naturally occurring gums and synthetic polymers such as polysaccharides (for example, acacia, agar, alginic acid, carrageenan, guar gum, karaya gum, and tragacanth), cellulose derivatives (for example, carboxymethylcellulose and carboxypropylcellulose), and synthetic polymers (for example, carbomers, cellulose ethers, and carboxyvinyl polymers). These disperse or swell in water to form colloidal solutions that stabilize emulsions by forming strong interfacial films around the dispersed-phase droplets and by increasing the viscosity of the external phase.

Since emulsions often contain a number of ingredients such as carbohydrates, proteins, sterols and phosphatides that can readily support the growth of microbes, these formulations often incorporate preservatives. Commonly used preservatives included in emulsion formulations include methyl paraben, propyl paraben, quaternary ammonium salts, benzalkonium chloride, esters of p-hydroxybenzoic acid, and boric acid. Antioxidants are also commonly added to emulsion formulations to prevent deterioration of the formulation. Antioxidants used can be free radical scavengers such as tocopherols, alkyl gallates, butylated hydroxyanisole, butylated hydroxytoluene, or reducing agents such as ascorbic acid and sodium metabisulfite, and antioxidant synergists such as citric acid, tartaric acid, and lecithin.

The application of emulsion formulations via dermatological, oral, and parenteral routes, and methods for their manufacture have been reviewed in the literature (see e.g., Ansel's Pharmaceutical Dosage Forms and Drug Delivery

Systems, Allen, L V., Popovich N G., and Ansel H C., 2004, Lippincott Williams & Wilkins (8th ed.), New York, N.Y.; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199). Emulsion formulations for oral delivery have been very widely used because of ease of formulation, as well as efficacy from an absorption and bioavailability standpoint (see e.g., Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, L V., Popovich N G., and Ansel H C., 2004, Lippincott Williams & Wilkins (8th ed.), New York, N.Y.; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199). Mineral-oil base laxatives, oil-soluble vitamins, and high fat nutritive preparations are among the materials that have commonly been administered orally as o/w emulsions.

ii. Microemulsions

In one embodiment of the present invention, the iRNAs are formulated as microemulsions. A microemulsion can be defined as a system of water, oil, and amphiphile which is a single optically isotropic and thermodynamically stable liquid solution (see e.g., Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, L V., Popovich N G., and Ansel H C., 2004, Lippincott Williams & Wilkins (8th ed.), New York, N.Y.; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245). Typically microemulsions are systems that are prepared by first dispersing an oil in an aqueous surfactant solution and then adding a sufficient amount of a fourth component, generally an intermediate chain-length alcohol to form a transparent system. Therefore, microemulsions have also been described as thermodynamically stable, isotropically clear dispersions of two immiscible liquids that are stabilized by interfacial films of surface-active molecules (Leung and Shah, in: Controlled Release of Drugs: Polymers and Aggregate Systems, Rosoff, M., Ed., 1989, VCH Publishers, New York, pages 185-215). Microemulsions commonly are prepared via a combination of three to five components that include oil, water, surfactant, cosurfactant and electrolyte. Whether the microemulsion is of the water-in-oil (w/o) or an oil-in-water (o/w) type is dependent on the properties of the oil and surfactant used and on the structure and geometric packing of the polar heads and hydrocarbon tails of the surfactant molecules (Schott, in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., 1985, p. 271).

The phenomenological approach utilizing phase diagrams has been extensively studied and has yielded a comprehensive knowledge, to one skilled in the art, of how to formulate microemulsions (see e.g., Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems, Allen, L V., Popovich N G., and Ansel H C., 2004, Lippincott Williams & Wilkins (8th ed.), New York, N.Y.; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245; Block, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335). Compared to conventional emulsions, microemulsions offer the advantage of solubilizing water-insoluble drugs in a formulation of thermodynamically stable droplets that are formed spontaneously.

Surfactants used in the preparation of microemulsions include, but are not limited to, ionic surfactants, non-ionic surfactants, Brij® 96, polyoxyethylene oleyl ethers, polyglycerol fatty acid esters, tetraglycerol monolaurate (ML310), tetraglycerol monooleate (MO310), hexaglycerol monooleate (PO310), hexaglycerol pentaoleate (PO500), decaglycerol monocaprate (MCA750), decaglycerol monooleate (MO750), decaglycerol sequioleate (SO750), decaglycerol decaoleate (DAO750), alone or in combination with cosurfactants. The cosurfactant, usually a short-chain alcohol such as ethanol, 1-propanol, and 1-butanol, serves to increase the interfacial fluidity by penetrating into the surfactant film and consequently creating a disordered film because of the void space generated among surfactant molecules. Microemulsions can, however, be prepared without the use of cosurfactants and alcohol-free self-emulsifying microemulsion systems are known in the art. The aqueous phase can typically be, but is not limited to, water, an aqueous solution of the drug, glycerol, PEG300, PEG400, polyglycerols, propylene glycols, and derivatives of ethylene glycol. The oil phase can include, but is not limited to, materials such as Captex® 300, Captex® 355, Capmul® MCM, fatty acid esters, medium chain (C8-C12) mono, di, and tri-glycerides, polyoxyethylated glyceryl fatty acid esters, fatty alcohols, polyglycolized glycerides, saturated polyglycolized C8-C10 glycerides, vegetable oils, and silicone oil.

Microemulsions are particularly of interest from the standpoint of drug solubilization and the enhanced absorption of drugs. Lipid based microemulsions (both o/w and w/o) have been proposed to enhance the oral bioavailability of drugs, including peptides (see e.g., U.S. Pat. Nos. 6,191,105; 7,063,860; 7,070,802; 7,157,099; Constantinides et al., Pharmaceutical Research, 1994, 11, 1385-1390; Ritschel, Meth. Find. Exp. Clin. Pharmacol., 1993, 13, 205). Microemulsions afford advantages of improved drug solubilization, protection of drug from enzymatic hydrolysis, possible enhancement of drug absorption due to surfactant-induced alterations in membrane fluidity and permeability, ease of preparation, ease of oral administration over solid dosage forms, improved clinical potency, and decreased toxicity (see e.g., U.S. Pat. Nos. 6,191,105; 7,063,860; 7,070,802; 7,157,099; Constantinides et al., Pharmaceutical Research, 1994, 11, 1385; Ho et al., J. Pharm. Sci., 1996, 85, 138-143). Often microemulsions can form spontaneously when their components are brought together at ambient temperature. This can be particularly advantageous when formulating thermolabile drugs, peptides or iRNAs. Microemulsions have also been effective in the transdermal delivery of active components in both cosmetic and pharmaceutical applications. It is expected that the microemulsion compositions and formulations of the present invention will facilitate the increased systemic absorption of iRNAs and nucleic acids from the gastrointestinal tract, as well as improve the local cellular uptake of iRNAs and nucleic acids.

Microemulsions of the present invention can also contain additional components and additives such as sorbitan monostearate (Grill® 3), Labrasol®, and penetration enhancers to improve the properties of the formulation and to enhance the absorption of the iRNAs and nucleic acids of the present invention. Penetration enhancers used in the microemulsions of the present invention can be classified as belonging to one of five broad categories—surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p. 92). Each of these classes has been discussed above.

iii. Microparticles

An iRNA of the invention may be incorporated into a particle, e.g., a microparticle. Microparticles can be pro-

duced by spray-drying, but may also be produced by other methods including lyophilization, evaporation, fluid bed drying, vacuum drying, or a combination of these techniques.

iv. Penetration Enhancers

In one embodiment, the present invention employs various penetration enhancers to effect the efficient delivery of nucleic acids, particularly iRNAs, to the skin of animals. Most drugs are present in solution in both ionized and nonionized forms. However, usually only lipid soluble or lipophilic drugs readily cross cell membranes. It has been discovered that even non-lipophilic drugs can cross cell membranes if the membrane to be crossed is treated with a penetration enhancer. In addition to aiding the diffusion of non-lipophilic drugs across cell membranes, penetration enhancers also enhance the permeability of lipophilic drugs.

Penetration enhancers can be classified as belonging to one of five broad categories, i.e., surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants (see e.g., Malmsten, M. Surfactants and polymers in drug delivery, Informa Health Care, New York, N.Y., 2002; Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p. 92). Such compounds are well known in the art.

v. Carriers

Certain compositions of the present invention also incorporate carrier compounds in the formulation. As used herein, "carrier compound" or "carrier" can refer to a nucleic acid, or analog thereof, which is inert (i.e., does not possess biological activity per se) but is recognized as a nucleic acid by in vivo processes that reduce the bioavailability of a nucleic acid having biological activity by, for example, degrading the biologically active nucleic acid or promoting its removal from circulation. The coadministration of a nucleic acid and a carrier compound, typically with an excess of the latter substance, can result in a substantial reduction of the amount of nucleic acid recovered in the liver, kidney or other extracirculatory reservoirs, presumably due to competition between the carrier compound and the nucleic acid for a common receptor. For example, the recovery of a partially phosphorothioate dsRNA in hepatic tissue can be reduced when it is coadministered with polyinosinic acid, dextran sulfate, polycytidic acid or 4-acetamido-4'isothiocyano-stilbene-2,2'-disulfonic acid (Miyao et al., DsRNA Res. Dev., 1995, 5, 115-121; Takakura et al., DsRNA & Nucl. Acid Drug Dev., 1996, 6, 177-183.

vi. Excipients

In contrast to a carrier compound, a "pharmaceutical carrier" or "excipient" is a pharmaceutically acceptable solvent, suspending agent, or any other pharmacologically inert vehicle for delivering one or more nucleic acids to an animal. The excipient can be liquid or solid and is selected, with the planned manner of administration in mind, so as to provide for the desired bulk, consistency, etc., when combined with a nucleic acid and the other components of a given pharmaceutical composition. Typical pharmaceutical carriers include, but are not limited to, binding agents (e.g., pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose, etc.); fillers (e.g., lactose and other sugars, microcrystalline cellulose, pectin, gelatin, calcium sulfate, ethyl cellulose, polyacrylates or calcium hydrogen phosphate, etc.); lubricants (e.g., magnesium stearate, talc, silica, colloidal silicon dioxide, stearic acid, metallic stearates, hydrogenated vegetable oils, corn starch, polyethylene glycols, sodium benzoate, sodium acetate, etc.); disintegrants (e.g., starch, sodium starch glycolate, etc.); and wetting agents (e.g., sodium lauryl sulphate, etc).

Pharmaceutically acceptable organic or inorganic excipients suitable for non-parenteral administration which do not deleteriously react with nucleic acids can also be used to formulate the compositions of the present invention. Suitable pharmaceutically acceptable carriers include, but are not limited to, water, salt solutions, alcohols, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose, polyvinylpyrrolidone, and the like.

Formulations for topical administration of nucleic acids can include sterile and non-sterile aqueous solutions, non-aqueous solutions in common solvents such as alcohols, or solutions of the nucleic acids in liquid or solid oil bases. The solutions can also contain buffers, diluents and other suitable additives. Pharmaceutically acceptable organic or inorganic excipients suitable for non-parenteral administration which do not deleteriously react with nucleic acids can be used.

Suitable pharmaceutically acceptable excipients include, but are not limited to, water, salt solutions, alcohol, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose, polyvinylpyrrolidone, and the like.

vii. Other Components

The compositions of the present invention can additionally contain other adjunct components conventionally found in pharmaceutical compositions, at their art-established usage levels. Thus, for example, the compositions can contain additional, compatible, pharmaceutically-active materials such as, for example, antipruritics, astringents, local anesthetics or anti-inflammatory agents, or can contain additional materials useful in physically formulating various dosage forms of the compositions of the present invention, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. However, such materials, when added, should not unduly interfere with the biological activities of the components of the compositions of the present invention. The formulations can be sterilized and, if desired, mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings or aromatic substances and the like which do not deleteriously interact with the nucleic acid(s) of the formulation.

Aqueous suspensions can contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol, or dextran. The suspension can also contain stabilizers.

In some embodiments, pharmaceutical compositions featured in the invention include (a) one or more iRNA and (b) one or more agents which function by a non-iRNA mechanism and which are useful in treating an XDH-associated disorder.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds that exhibit high therapeutic indices are preferred.

The data obtained from cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of compositions featured herein in the invention lies generally within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage can vary within this range depending

upon the dosage form employed and the route of administration utilized. For any compound used in the methods featured in the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range of the compound or, when appropriate, of the polypeptide product of a target sequence (e.g., achieving a decreased concentration of the polypeptide) that includes the IC50 (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma can be measured, for example, by high performance liquid chromatography.

In addition to their administration, as discussed above, the iRNAs featured in the invention can be administered in combination with other known agents effective in treatment of pathological processes mediated by XDH expression. In any event, the administering physician can adjust the amount and timing of iRNA administration on the basis of results observed using standard measures of efficacy known in the art or described herein.

VI. Methods For Inhibiting XDH Expression

The present invention also provides methods of inhibiting expression of an XDH gene in a cell. The methods include contacting a cell with an RNAi agent, e.g., double stranded RNAi agent, in an amount effective to inhibit expression of XDH in the cell, thereby inhibiting expression of XDH in the cell.

Contacting of a cell with an iRNA, e.g., a double stranded RNAi agent, may be done in vitro or in vivo. Contacting a cell in vivo with the iRNA includes contacting a cell or group of cells within a subject, e.g., a human subject, with the iRNA. Combinations of in vitro and in vivo methods of contacting a cell are also possible. Contacting a cell may be direct or indirect, as discussed above. Furthermore, contacting a cell may be accomplished via a targeting ligand, including any ligand described herein or known in the art. In preferred embodiments, the targeting ligand is a carbohydrate moiety, e.g., a $GalNAc_3$ ligand, or any other ligand that directs the RNAi agent to a site of interest.

The term "inhibiting," as used herein, is used interchangeably with "reducing," "silencing," "downregulating", "suppressing", and other similar terms, and includes any level of inhibition.

The phrase "inhibiting expression of an XDH" is intended to refer to inhibition of expression of any XDH gene (such as, e.g., a mouse XDH gene, a rat XDH gene, a monkey XDH gene, or a human XDH gene) as well as variants or mutants of an XDH gene. Thus, the XDH gene may be a wild-type XDH gene, a mutant XDH gene (such as a mutant XDH gene giving rise to abnormal uric acid metabolism), or a transgenic XDH gene in the context of a genetically manipulated cell, group of cells, or organism.

"Inhibiting expression of an XDH gene" includes any level of inhibition of an XDH gene, e.g., at least partial suppression of the expression of an XDH gene, inhibition of XDH in certain cells or tissues. The expression of the XDH gene may be assessed based on the level, or the change in the level, of any variable associated with XDH gene expression, e.g., XDH mRNA level or XDH protein level. This level may be assessed in an individual cell or in a group of cells, including, for example, a sample derived from a subject.

Inhibition may be assessed by a decrease in an absolute or relative level of one or more variables that are associated with XDH expression compared with a control level. The control level may be any type of control level that is utilized in the art, e.g., a pre-dose baseline level, or a level determined from a similar subject, cell, or sample that is untreated or treated with a control (such as, e.g., buffer only control or inactive agent control).

In some embodiments of the methods of the invention, expression of an XDH gene is inhibited by at least 15%, 20%, 25%, preferably at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%, or to below the level of detection of the assay. In some embodiments, the inhibition of expression of an XDH gene results in normalization of the level of the XDH gene such that the difference between the level before treatment and a normal control level is reduced by at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%. In some embodiments, the inhibition is a clinically relevant inhibition.

Inhibition of the expression of an XDH gene may be manifested by a reduction of the amount of mRNA expressed by a first cell or group of cells (such cells may be present, for example, in a sample derived from a subject) in which an XDH gene is transcribed and which has or have been treated (e.g., by contacting the cell or cells with an iRNA of the invention, or by administering an iRNA of the invention to a subject in which the cells are or were present) such that the expression of an XDH gene is inhibited, as compared to a second cell or group of cells substantially identical to the first cell or group of cells but which has not or have not been so treated (control cell(s) not treated with an iRNA or not treated with an iRNA targeted to the gene of interest). In preferred embodiments, the inhibition is assessed by the method provided in Example 2 with in vitro assays being performed in an appropriately matched cell line with the duplex at a 10 nM concentration, and expressing the level of mRNA in treated cells as a percentage of the level of mRNA in control cells, using the following formula:

$$\frac{(mRNA \text{ in control cells}) - (mRNA \text{ in treated cells})}{(mRNA \text{ in control cells})} \cdot 100\%$$

In other embodiments, inhibition of the expression of an XDH gene may be assessed in terms of a reduction of a parameter that is functionally linked to XDH gene expression, e.g., serum uric acid levels. XDH gene silencing may be determined in any cell expressing XDH, either endogenous or heterologous from an expression construct, and by any assay known in the art.

Inhibition of the expression of an XDH protein may be manifested by a reduction in the level of the XDH protein that is expressed by a cell or group of cells (e.g., the level of protein expressed in a sample derived from a subject). As explained above, for the assessment of mRNA suppression, the inhibition of protein expression levels in a treated cell or group of cells may similarly be expressed as a percentage of the level of protein in a control cell or group of cells. For example, inhibition of expression can be inhibition of expression in liver cells.

A control cell or group of cells that may be used to assess the inhibition of the expression of an XDH gene includes a cell or group of cells that has not yet been contacted with an RNAi agent of the invention. For example, the control cell or group of cells may be derived from an individual subject (e.g., a human or animal subject) prior to treatment of the subject with an RNAi agent.

The level of XDH mRNA that is expressed by a cell or group of cells, or the level of circulating XDH mRNA, may be determined using any method known in the art for assessing mRNA expression. In one embodiment, the level of expression of XDH in a sample is determined by detecting a transcribed polynucleotide, or portion thereof, e.g., mRNA of the XDH gene. RNA may be extracted from cells using RNA extraction techniques including, for example, using acid phenol/guanidine isothiocyanate extraction (RNAzol B; Biogenesis), RNeasy™ RNA preparation kits (Qiagen®) or PAXgene (PreAnalytix, Switzerland). Typical assay formats utilizing ribonucleic acid hybridization include nuclear run-on assays, RT-PCR, RNase protection assays, northern blotting, in situ hybridization, and microarray analysis. In preferred embodiments, the RT-PCR assay provided in Example 2, with in vitro assays being performed in an appropriately matched cell line with the duplex at a 10 nM concentration, is used to detect the level of expression of the mRNA.

In some embodiments, the level of expression of XDH is determined using a nucleic acid probe. The term "probe", as used herein, refers to any molecule that is capable of selectively binding to a specific XDH. Probes can be synthesized by one of skill in the art, or derived from appropriate biological preparations. Probes may be specifically designed to be labeled. Examples of molecules that can be utilized as probes include, but are not limited to, RNA, DNA, proteins, antibodies, and organic molecules.

Isolated mRNA can be used in hybridization or amplification assays that include, but are not limited to, Southern or northern analyses, polymerase chain reaction (PCR) analyses and probe arrays. One method for the determination of mRNA levels involves contacting the isolated mRNA with a nucleic acid molecule (probe) that can hybridize to XDH mRNA. In one embodiment, the mRNA is immobilized on a solid surface and contacted with a probe, for example by running the isolated mRNA on an agarose gel and transferring the mRNA from the gel to a membrane, such as nitrocellulose. In an alternative embodiment, the probe(s) are immobilized on a solid surface and the mRNA is contacted with the probe(s), for example, in an Affymetrix gene chip array. A skilled artisan can readily adapt known mRNA detection methods for use in determining the level of XDH mRNA.

An alternative method for determining the level of expression of XDH in a sample involves the process of nucleic acid amplification or reverse transcriptase (to prepare cDNA) of for example mRNA in the sample, e.g., by RT-PCR (the experimental embodiment set forth in Mullis, 1987, U.S. Pat. No. 4,683,202), ligase chain reaction (Barany (1991) *Proc. Natl. Acad. Sci. USA* 88:189-193), self sustained sequence replication (Guatelli et al. (1990) *Proc. Natl. Acad. Sci. USA* 87:1874-1878), transcriptional amplification system (Kwoh et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:1173-1177), Q-Beta Replicase (Lizardi et al. (1988) *Bio/Technology* 6:1197), rolling circle replication (Lizardi et al., U.S. Pat. No. 5,854,033) or any other nucleic acid amplification method, followed by the detection of the amplified molecules using techniques well known to those of skill in the art. These detection schemes are especially useful for the detection of nucleic acid molecules if such molecules are present in very low numbers. In particular aspects of the invention, the level of expression of XDH is determined by quantitative fluorogenic RT-PCR (i.e., the TaqMan™ System) using the methods provided herein.

The expression levels of XDH mRNA may be monitored using a membrane blot (such as used in hybridization analysis such as northern, Southern, dot, and the like), or microwells, sample tubes, gels, beads or fibers (or any solid support comprising bound nucleic acids). See U.S. Pat. Nos. 5,770,722, 5,874,219, 5,744,305, 5,677,195 and 5,445,934, which are incorporated herein by reference. The determination of XDH expression level may also comprise using nucleic acid probes in solution.

In preferred embodiments, the level of mRNA expression is assessed using branched DNA (bDNA) assays or real time PCR (qPCR). The use of these methods is described and exemplified in the Examples presented herein.

The level of XDH protein expression may be determined using any method known in the art for the measurement of protein levels. Such methods include, for example, electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), hyperdiffusion chromatography, fluid or gel precipitin reactions, absorption spectroscopy, a colorimetric assays, spectrophotometric assays, flow cytometry, immunodiffusion (single or double), immunoelectrophoresis, western blotting, radioimmunoassay (RIA), enzyme-linked immunosorbent assays (ELISAs), immunofluorescent assays, electrochemiluminescence assays, and the like.

In some embodiments, the efficacy of the methods of the invention in the treatment of an XDH-related disease is assessed by a decrease in XDH mRNA level (by liver biopsy) or XDH protein level, typically determined in serum.

In some embodiments, the efficacy of the compositions methods of the invention in the treatment of hyperuricemia is demonstated by a significant decrease in chronic uric acid levels in a subject to 6.8 mg/dl or less (the level of solubility of uric acid in serum), preferably 6 mg/dl or less. Studies of subjects with inherited disorders associated with profound, lifelong hypouricemia indicate that maintaining serum uric acid near or below 2 mg/dl is safe (Hershfeld, *Curr. Opin. Rheumatol.* 21:138-142, 2009). As elevated levels of serum uric acid are associated with a number of diseases and conditions, a decrease in chronic uric acid levels towards, or preferably to, normal levels, would treat those conditions. The compositions and methods of the invention can also be used to treat aymptomatic hyperuricemia. Normalizing hyperuricemia prevents one or more of the comorbidities associated with hyperuricemia including, but not limited to, gout, NAFLD, NASH, metabolic disorder, insulin resistance, cardiovascular disease, hypertension, type 2 diabetes, and conditions linked to oxidative stress e.g., chronic low grade inflammation; or other XDH-associated disease. In some embodiments of the methods of the invention, the iRNA is administered to a subject such that the iRNA is delivered to a specific site within the subject. The inhibition of expression of XDH may be assessed using measurements of the level or change in the level of XDH mRNA or XDH protein in a sample derived from a specific site within the subject, e.g., the liver.

As used herein, the terms detecting or determining a level of an anlyte are understood to mean performing the steps to determine if a material, e.g., protein, RNA, is present. As used herein, methods of detecting or determining include detection or determination of an anlyte level that is below the level of detection for the method used.

VII. Methods of Treating or Preventing XDH-Associated Diseases

The present invention provides therapeutic and prophylactic methods which include administering to a subject

having an XDH-associated disease, disorder, and/or condition, or prone to developing, an XDH-associated disease, disorder, and/or condition, compositions comprising an iRNA agent targeting an XDH gene. Non-limiting examples of XDH-associated diseases include, for example, gout, NAFLD, NASH, metabolic disorder, insulin resistance, cardiovascular disease, hypertension, type 2 diabetes, and conditions linked to oxidative stress e.g., chronic low grade inflammation.

The methods of the invention are useful for treating a subject having an XDH-associated disease, e.g., a subject that would benefit from reduction in XDH gene expression and/or XDH protein production.

In one aspect, the invention provides methods of preventing at least one sign or symptom in a subject susceptible to or having an XDH-associated disease, e.g., gout, NAFLD, NASH, metabolic disorder, insulin resistance, cardiovascular disease, hypertension, type 2 diabetes, and conditions linked to oxidative stress e.g., chronic low grade inflammation. The methods include administering to the subject a prohylactically effective amount of the iRNA agent, e.g. dsRNA, pharmaceutical compositions, or vectors of the invention, thereby preventing at least one sign or symptom in a subject having an XDH-associated disease.

In one embodiment, an iRNA agent targeting XDH is administered to a subject having an XDH-associated disease such that the expression of an XDH gene, e.g., in a cell, tissue, blood or other tissue or fluid of the subject are reduced by at least about 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29%, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 51%, 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, 60%, 61%, 62%, 62%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least about 99% or more, or to a level below the level of detection of the assay, when the dsRNA agent is administered to the subject.

The methods and uses of the invention include administering a composition described herein such that expression of the target XDH gene is decreased for an extended duration, e.g., at least one month, preferably at least three months.

Administration of the dsRNA according to the methods and uses of the invention may result in a reduction of the severity, signs, symptoms, and/or markers of such diseases or disorders in a patient with an XDH-associated disease. By "reduction" in this context is meant a statistically or clinically significant decrease in such level. The reduction can be, for example, at least about 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or about 100%.

Efficacy of treatment or prevention of disease can be assessed, for example by measuring disease progression, disease remission, symptom severity, reduction in pain, quality of life, dose of a medication required to sustain a treatment effect, level of a disease marker or any other measurable parameter appropriate for a given disease being treated or targeted for prevention. It is well within the ability of one skilled in the art to monitor efficacy of treatment or prevention by measuring any one of such parameters, or any combination of parameters. Comparison of the later readings with the initial readings, or historically relevant population controls, provide a physician an indication of whether the treatment is effective. It is well within the ability of one skilled in the art to monitor efficacy of treatment or prevention by measuring any one of such parameters, or any combination of parameters. In connection with the administration of an iRNA targeting an XDH gene or pharmaceutical composition thereof, "effective against" an XDH-associated disease indicates that administration in a clinically appropriate manner results in a beneficial effect for at least a statistically significant fraction of patients, such as improvement of symptoms, a cure, a reduction in disease, extension of life, improvement in quality of life, or other effect generally recognized as positive by medical doctors familiar with treating an XDH-associated disease and the related causes.

A treatment or preventive effect is evident when there is a statistically significant improvement in one or more parameters of disease status, or by a failure to worsen or to develop symptoms where they would otherwise be anticipated. As an example, a favorable change of at least 10% in a measurable parameter of disease, and preferably at least 20%, 30%, 40%, 50% or more can be indicative of effective treatment. Efficacy for a given iRNA drug or formulation of that drug can also be judged using an experimental animal model for the given disease as known in the art. When using an experimental animal model, efficacy of treatment is evidenced when a statistically significant reduction in a marker or symptom is observed.

XDH expression may be inhibited, e.g., in a liver cell of a subject, by at least 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, or 95, or to a level below the level of detection of the assay. The methods of the invention do not require knockdown of expression in all cell types in which XDH is expressed.

The in vivo methods of the invention may include administering to a subject a composition containing an iRNA, where the iRNA includes a nucleotide sequence that is complementary to at least a part of an RNA transcript of the XDH gene of the mammal to be treated. When the organism to be treated is a mammal such as a human, the composition can be administered by any means known in the art including, but not limited to oral, intraperitoneal, or parenteral routes, including intravenous, intramuscular, subcutaneous, transdermal, airway (aerosol), nasal, rectal, and topical (including buccal and sublingual) administration. In certain embodiments, the compositions are administered by intravenous infusion or injection. In certain embodiments, the compositions are administered by subcutaneous injection.

In some embodiments, the administration is via a depot injection. A depot injection may release the iRNA in a consistent way over a prolonged time period. Thus, a depot injection may reduce the frequency of dosing needed to obtain a desired effect, e.g., a desired inhibition of XDH, or a therapeutic or prophylactic effect. A depot injection may also provide more consistent serum concentrations. Depot injections may include subcutaneous injections or intramuscular injections. In preferred embodiments, the depot injection is a subcutaneous injection.

In some embodiments, the administration is via a pump. The pump may be an external pump or a surgically implanted pump. In certain embodiments, the pump is a subcutaneously implanted osmotic pump. In other embodiments, the pump is an infusion pump. An infusion pump may be used for intravenous, subcutaneous, arterial, or epidural infusions. In preferred embodiments, the infusion pump is a subcutaneous infusion pump. In other embodiments, the pump is a surgically implanted pump that delivers the iRNA to the liver.

The mode of administration may be chosen based upon whether local or systemic treatment is desired and based upon the area to be treated. The route and site of administration may be chosen to enhance targeting.

In one aspect, the present invention also provides methods for inhibiting the expression of an XDH gene in a mammal. The methods include administering to the mammal a composition comprising an iRNA that targets an XDH gene in a cell of the mammal and maintaining the mammal for a time sufficient to obtain degradation of the mRNA transcript of the XDH gene, thereby inhibiting expression of the XDH gene in the cell. Reduction in gene expression can be assessed by any methods known it the art and by methods, e.g. qRT-PCR, described herein. Reduction in protein production can be assessed by any methods known it the art and by methods, e.g. ELISA, described herein. In one embodiment, a puncture liver biopsy sample serves as the tissue material for monitoring the reduction in the XDH gene or protein expression.

The present invention further provides methods of treatment of a subject in need thereof. The treatment methods of the invention include administering an iRNA of the invention to a subject, e.g., a subject that would benefit from a reduction or inhibition of XDH expression, in a therapeutically effective amount of an iRNA targeting an XDH gene or a pharmaceutical composition comprising an iRNA targeting an XDH gene.

An iRNA of the invention may be administered as a "free iRNA." A free iRNA is administered in the absence of a pharmaceutical composition. The naked iRNA may be in a suitable buffer solution. The buffer solution may comprise acetate, citrate, prolamine, carbonate, or phosphate, or any combination thereof. In one embodiment, the buffer solution is phosphate buffered saline (PBS). The pH and osmolarity of the buffer solution containing the iRNA can be adjusted such that it is suitable for administering to a subject.

Alternatively, an iRNA of the invention may be administered as a pharmaceutical composition, such as a dsRNA liposomal formulation.

Subjects that would benefit from a reduction or inhibition of XDH gene expression are those having hyperuricemia as demonstrated by a chronic uric acid level of at least 6.8 mg/dl. It is expected that normalizing hyperuricemia would prevent one or more of the comorbidities associated with hyperuricemia including, but not limited to, gout, NAFLD, NASH, metabolic disorder, insulin resistance, cardiovascular disease, hypertension, type 2 diabetes, and conditions linked to oxidative stress e.g., chronic low grade inflammation; or other XDH-associated disease.

A. Hyperuricemia

Serum uric acid levels are not routinely obtained as clinical lab values. However, hyperuricemia is associated with a number of diseases and conditions including, gout, NAFLD, NASH, metabolic disorder, insulin resistance, cardiovascular disease, hypertension, type 2 diabetes, and conditions linked to oxidative stress e.g., chronic low grade inflammation. Normalization of uric acid levels is useful in the prevention or treatment of one or more conditions associated with elevated serum uric acid levels. Further, a subject derives clinical benefit from normalization of serum uric acid levels towards or to a normal serum uric acid level, e.g., no more than 6.8 mg/dl, preferably no more than 6 mg/dl, even in the absence of overt signs or symptoms of one or more conditions associated with elevated uric acid, e.g., gout, NAFLD, NASH, metabolic disorder, insulin resistance, cardiovascular disease, hypertension, type 2 diabetes, or conditions linked to oxidative stress e.g., chronic low grade inflammation. Methods to detect and monitor uric acid in serum or other subject samples are known in the art. Uric acid levels can be detected, for example using carbonate-phosphotungstate method, spectrophotometric uricase method, or chromatotgraphy methods such as HPLC or LCMS.

Allopurinol is a xanthine oxidase inhibitor that is used to reduce serum uric acid levels for the treatment of a number of conditions, e.g., gout, cardiovascular disease including ischemia-reperfusion injury, hypertension, atherosclerosis, and stroke, and inflammatory diseases (Pacher et al., Pharma. Rev. 58:87-114, 2006). However, the use of allopurinol is contraindicated in subjects with impaired renal function, e.g., chronic kidney disease, hypothyroidism, hyperinsulinemia, or insulin resistance; or in subjects predisposed to kidney disease or impaired renal function, e.g., subjects with hypertension, metabolic disorder, diabetes, and the elderly. Further, allopurinol should not be taken by subjects taking oral coagulants or probencid as well as subjects taking diuretics, especially thiazide diuretics or other drugs that can reduce kidney function or have potential kidney toxicity.

In certain embodiments, the compositions and methods of the invention are for use in treatment of subjects with hyperuricemia and impaired renal function. For example, in certain embodiments, the compositions and methods of the invention are for use in subjects with hyperuricemia and chronic kidney disease. In certain embodiments, the compositions and methods are for use in subjects with hyperuricemia who are suffering from one or more of cardiovascular disease, metabolic disorder, insulin resistance, hyperinsulinemia, diabetes, hypothyroidism, or inflammatory disease; or elderly subjects (e.g., over 65). In certain embodiments, the compositions and methods are for use in subjects with hyperuricemia who are also taking a drug that can reduce kidney function as demonstrated by the drug label. For example, in certain embodiments the compositions and methods of the invention are for use in subjects with hyperuricemia who are being treated with oral coagulants or probencid. For example, in certain embodiments the compositions and methods of the invention are for use in subjects with hyperuricemia who are being treated with diuretics, especially thiazide diuretics.

In certain embodiments, the compositions and methods of the invention are used in combination with other compositions and methods to treat hyperuricemia, e.g., allopurinol, oxypurinol, febuxostat.

B. Gout

Gout affects approximately 1 in 40 adults, most commonly men between 30-60 years of age. Gout less commonly affects women. Gout is one of a few types of arthritis where future damage to joints can be avoided by treatment. Gout is characterized by recurrent attacks of acute inflammatory arthritis caused by an inflammatory reaction to uric acid crystals in the joint due to hyperuricemia resulting from insufficient renal clearance of uric acid or excessive uric acid production. Fructose associated gout is associated with variants of transporters expressed in the kidney, intestine, and liver. Gout is characterized by the formation and deposition of tophi, monosodium urate (MSU) crystals, in the joints and subcutaneously. Pain associated with gout is not related to the size of the tophi, but is a result of an immune response against the MSU crystals. There is a linear inverse relation between serum uric acid and the rate of decrease in tophus size. For example, in one study of 18 patients with non-tophaceous gout, serum uric acid declined to 2.7-5.4 mg/dL (0.16-0.32 mM) in all subjects within 3 months of

starting urate lowering therapy (Pascual and Sivera, Ann. Rheum. Dis. 66:1056-1058). However, it took 12 months with normalized serum uric acid for MSU crystals to disappear from asymptomatic knee or first MTP joints in patients who had gout for less than 10 years, vs. 18 months in those with gout for more than 10 years. Therefore, effective treatment of gout does not require complete clearance of tophi or resolution of all symptoms, e.g., joint pain and swelling, inflammation, but simply a reduction in at least one sign or symptom of gout, e.g., reduction in severity or frequency of gout attacks, in conjunction with a reduction in serum urate levels.

Currently available treatments for gout are contraindicated or ineffective in a number of subjects. Allopurinol, a common first line treatment to reduce uric acid levels in subjects with gout, is contraindicated in a number of populations, especially those with compromised renal function, as discussed above. Further, a number of subjects fail treatment with allopurinol, e.g., subjects who suffer gout flares despite treatment, or subjects who suffer from rashes or hypersensitivity reactions associated with allopurinol.

In certain embodiments, the compositions and methods of the invention are for use in treatment of subjects with gout and impaired renal function. For example, in certain embodiments, the compositions and methods of the invention are for use in subjects with gout and chronic kidney disease. In certain embodiments, the compositions and methods are for use in subjects with gout who are suffering from one or more of cardiovascular disease, metabolic disorder, insulin resistance, hyperinsulinemia, diabetes, hypothyroidism, or inflammatory disease; or elderly subjects (e.g., over 65). In certain embodiments, the compositions and methods are for use in subjects with gout who are also taking a drug that can reduce kidney function as demonstrated by the drug label. For example, in certain embodiments the compositions and methods of the invention are for use in subjects with gout who are being treated with oral coagulants or probencid. For example, in certain embodiments the compositions and methods of the invention are for use in subjects with gout who are being treated with diuretics, especially thiazide diuretics. For example, in certain embodiments the compositions and methods of the invention are for use in subjects with gout who have failed treatment with allopurinol.

In certain embodiments, the compositions and methods of the invention are used in combination with other agents to reduce serum uric acid. In certain embodiments, the compositions and methods of the invention are used in combination with agents for treatment of symptoms of gout, e.g., analgesic or anti-inflammatory agents, e.g., NSAIDS.

C. NAFLD

NAFLD is associated with hyperuricemia (Xu et al., J. Hepatol. 62:1412-1419, 2015). The definition of nonalcoholic fatty liver disease (NAFLD) requires that (a) there is evidence of hepatic steatosis, either by imaging or by histology and (b) there are no causes for secondary hepatic fat accumulation such as significant alcohol consumption, use of steatogenic medication or hereditary disorders. In the majority of patients, NAFLD is associated with metabolic risk factors such as obesity, diabetes mellitus, and dyslipidemia. NAFLD is histologically further categorized into nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH). NAFL is defined as the presence of hepatic steatosis with no evidence of hepatocellular injury in the form of ballooning of the hepatocytes. NASH is defined as the presence of hepatic steatosis and inflammation with hepatocyte injury (ballooning) with or without fibrosis (Chalasani et al., Hepatol. 55:2005-2023, 2012). It is generally agreed that patients with simple steatosis have very slow, if any, histological progression, while patients with NASH can exhibit histological progression to cirrhotic-stage disease. The long term outcomes of patients with NAFLD and NASH have been reported in several studies. Their findings can be summarized as follows; (a) patients with NAFLD have increased overall mortality compared to matched control populations, (b) the most common cause of death in patients with NAFLD, NAFL, and NASH is cardiovascular disease, and (c) patients with NASH (but not NAFL) have an increased liver-related mortality rate. In a mouse model of NAFLD, treatment with allopurinol both prevented the development of hepatic steatosis, but also significantly ameliorated established hepatic steatosis in mice (Xu et al., J. Hepatol. 62:1412-1419, 2015).

As discussed above, treatment with allopurinol is contraindicated in a number of populations, especially those with compromised renal function.

In certain embodiments, the compositions and methods of the invention are for use in treatment of subjects with NAFLD and impaired renal function. For example, in certain embodiments, the compositions and methods of the invention are for use in subjects with NAFLD and chronic kidney disease. In certain embodiments, the compositions and methods are for use in subjects with NAFLD who are suffering from one or more of cardiovascular disease, metabolic disorder, insulin resistance, hyperinsulinemia, diabetes, hypothyroidism, or inflammatory disease; or elderly subjects (e.g., over 65). In certain embodiments, the compositions and methods are for use in subjects with NAFLD who are also taking a drug that can reduce kidney function as demonstrated by the drug label. For example, in certain embodiments the compositions and methods of the invention are for use in subjects with NAFLD who are being treated with oral coagulants or probencid. For example, in certain embodiments the compositions and methods of the invention are for use in subjects with NAFLD who are being treated with diuretics, especially thiazide diuretics. For example, in certain embodiments the compositions and methods of the invention are for use in subjects with NAFLD who have failed treatment with allopurinol.

In certain embodiments, the compositions and methods of the invention are used in combination with other agents to reduce serum uric acid. In certain embodiments, the compositions and methods of the invention are used in combination with agents for treatment of symptoms of NAFLD.

D. Cardiovascular disease

Cardiovascular disease has been associated with hyperuricemia. Allopurinol has been demonstrated to be effective in the treatment of cardiovascular disease in animal models and humans including myocardial infarction, ischemia-reperfusion injury, hypoxia, ischemic heart disease, heart failure, hypercholesterolemia, and hypertension (Pacher et al., Pharma. Rev. 58:87-114, 2006). As discussed above, treatment with allopurinol is contraindicated in a number of populations, especially those with compromised renal function.

In certain embodiments, the compositions and methods of the invention are for use in treatment of subjects with cardiovascular disease and impaired renal function. For example, in certain embodiments, the compositions and methods of the invention are for use in subjects with cardiovascular disease and chronic kidney disease. In certain embodiments, the compositions and methods are for use in subjects with cardiovascular disease who are suffering from one or more of metabolic disorder, insulin resistance, hyper-

insulinemia, diabetes, hypothyroidism, or inflammatory disease. In certain embodiments, the compositions and methods are for use in subjects with cardiovascular disease who are also taking a drug that can reduce kidney function as demonstrated by the drug label. For example, in certain embodiments the compositions and methods of the invention are for use in subjects with cardiovascular disease who are being treated with oral coagulants or probencid. For example, in certain embodiments the compositions and methods of the invention are for use in subjects with cardiovascular disease who are being treated with diuretics, especially thiazide diuretics. For example, in certain embodiments the compositions and methods of the invention are for use in subjects with cardiovascular disease who have failed treatment with allopurinol.

In certain embodiments, the compositions and methods of the invention are used in combination with other agents to reduce serum uric acid. In certain embodiments, the compositions and methods of the invention are used in combination with agents for treatment of symptoms of cardiovascular disease, e.g., agents to decrease blood pressure, e.g., diuretics, beta-blockers, ACE inhibitors, angiotensin II receptor blockers, calcium channel blockers, alpha blockers, alpha-2 receptor antagonists, combined alpha- and beta-blockers, central agonists, peripheral adrenergic inhibitors, and blood vessel dialators; or agents to decrease cholesterol, e.g., statins, selective cholesterol absorption inhibitors, resins, or lipid lowering therapies.

E. Metabolic Syndrome, Insulin Resistance, and Type 2 Diabetes

Metabolic syndrome, insulin resistance, and type 2 diabetes are associated with hyperuricemia (Cardoso et al., *J. Pediatr.* 89:412-418, 2013).

Metabolic syndrome is characterized by a cluster of conditions defined as at least three of the five following metabolic risk factors:

1. Large waistline (≥35 inches for women or ≥40 inches for men);
2. High triglyceride level (≥150 mg/dl);
3. Low HDL cholesterol (≤50 mg/dl for women or ≤40 mg/dl for men);
4. Elevated blood pressure (≥130/85) or on medicine to treat high blood pressure; and
5. High fasting blood sugar (≥100 mg/dl) or being in medicine to treat high blood sugar.

Insulin resistance is characterized by the presence of at least one of:

1. A fasting blood glucose level of 100-125 mg/dL taken at two different times; or
2. An oral glucose tolerance test with a result of a glucose level of 140-199 mg/dL at 2 hours after glucose consumption.

Type 2 diabetes is characterized by at least one of:

1. A fasting blood glucose level ≥126 mg/dL taken at two different times;
2. A hemoglobin A1c (A1C) test with a result of ≥6.5% or higher; or
3. An oral glucose tolerance test with a result of a glucose level ≥200 mg/dL at 2 hours after glucose consumption.

Metabolic syndrome, insulin resistance, and type 2 diabetes are often associated with decreased renal function or the potential for decreased renal function.

In certain embodiments, the compositions and methods of the invention are for use in treatment of subjects with metabolic syndrome, insulin resistance, or type 2 diabetes and impaired renal function. For example, in certain embodiments, the compositions and methods of the invention are for use in subjects with metabolic syndrome, insulin resistance, or type 2 diabetes and chronic kidney disease. In certain embodiments, the compositions and methods are for use in subjects with metabolic syndrome, insulin resistance, or type 2 diabetes who are suffering from one or more of cardiovascular disease, hypothyroidism, or inflammatory disease; or elderly subjects (e.g., over 65). In certain embodiments, the compositions and methods are for use in subjects with metabolic syndrome, insulin resistance, or type 2 diabetes who are also taking a drug that can reduce kidney function as demonstrated by the drug label. For example, in certain embodiments the compositions and methods of the invention are for use in subjects with metabolic syndrome, insulin resistance, or type 2 diabetes who are being treated with oral coagulants or probencid. For example, in certain embodiments the compositions and methods of the invention are for use in subjects with metabolic syndrome, insulin resistance, or type 2 diabetes who are being treated with diuretics, especially thiazide diuretics.

In certain embodiments, the compositions and methods of the invention are used in combination with other agents to reduce serum uric acid. In certain embodiments, the compositions and methods of the invention are used in combination with agents for treatment of symptoms of metabolic syndrome, insulin resistance, or type 2 diabetes. In certain embodiments, subjects are treated with e.g., agents to decrease blood pressure, e.g., diuretics, beta-blockers, ACE inhibitors, angiotensin II receptor blockers, calcium channel blockers, alpha blockers, alpha-2 receptor antagonists, combined alpha- and beta-blockers, central agonists, peripheral adrenergic inhibitors, and blood vessel dialators; agents to decrease cholesterol, e.g., statins, selective cholesterol absorption inhibitors, resins, or lipid lowering therapies; or agents to normalize blood sugar, e.g., metformin, sulfonylureas, meglitinides, thiazolidinediones, DPP-4 inhibitors, GLP-1 receptor antagonists, and SGLT2 inhibitors.

The iRNA and additional therapeutic agents may be administered at the same time or in the same combination, e.g., parenterally, or the additional therapeutic agent can be administered as part of a separate composition or at separate times or by another method known in the art or described herein.

In one embodiment, the method includes administering a composition featured herein such that expression of the target XDH gene is decreased in at least one tissue, e.g., in liver, such as for about 1, 2, 3, 4, 5, 6, 7, 8, 12, 16, 18, 24 hours, 28, 32, or about 36 hours. In one embodiment, expression of the target XDH gene is decreased for an extended duration, e.g., at least about two, three, four days or more, e.g., about one week, two weeks, three weeks, or four weeks or longer, in at least one tissue, e.g., liver.

Preferably, the iRNAs useful for the methods and compositions featured herein specifically target RNAs (primary or processed) of the target XDH gene. Compositions and methods for inhibiting the expression of these genes using iRNAs can be prepared and performed as described herein.

Administration of the iRNA according to the methods of the invention may result in a reduction of the severity, signs, symptoms, or markers of such diseases or disorders in a patient with a disorder of elevated serum uric acid. By "reduction" in this context is meant a statistically significant decrease in such level. The reduction can be, for example, at least about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%, or to below the level of detection of the assay used. For certain measures, a reduction of a marker to the lowest possible level may not be desirable. For example, as used herein, reduction

includes a lowering towards or to a normal serum uric acid level in the subject. For example, reduction includes lowering towards or preferably to 6.8 mg/dl or less of serum uric acid. In certain embodiments, reduction includes lowering to 6 mg/dl or less of serum uric acid. Typically, a serum uric acid level of at least 2 mg/dl is preferred. Similarly, triglyceride levels are preferably lowered (i.e., normalized) to less than 150 mg/dl and fasting blood sugar is lowered (i.e., normalized) to less than 100 mg/dl. Such considerations are well understood by those of skill in the art.

Efficacy of treatment or prevention of disease can be assessed, for example by measuring disease progression, disease remission, symptom severity, reduction in pain, quality of life, dose of a medication required to sustain a treatment effect, level of a disease marker, or any other measurable parameter appropriate for a given disease being treated or targeted for prevention. It is well within the ability of one skilled in the art to monitor efficacy of treatment or prevention by measuring any one of such parameters, or any combination of parameters. For example, efficacy of treatment of an XDH related disorder. Diagnostic criteria for a number of XDH related disorders are provided above. The exact criteria for treatment or prevention will depend on the disease or condition present in the subject or the disease or condition to which the subject is susceptible. Treatment and prevention typically include normalization or maintenance of normal clinical laboratory values.

Comparisons of the later readings with the initial readings provide a physician an indication of whether the treatment is effective. It is well within the ability of one skilled in the art to monitor efficacy of treatment or prevention by measuring any one of such parameters, or any combination of parameters. In connection with the administration of an iRNA targeting XDH or pharmaceutical composition thereof, "effective against" an XDH related disorder indicates that administration in a clinically appropriate manner results in a beneficial effect for at least a statistically significant fraction of patients, such as a improvement of symptoms, a cure, a reduction in disease, extension of life, improvement in quality of life, or other effect generally recognized as positive by medical doctors familiar with treating XDH-related disorders.

A treatment or preventive effect is evident when there is a statistically significant improvement in one or more parameters of disease status, or by a failure to worsen or to develop symptoms where they would otherwise be anticipated. As an example, a favorable change of at least 10% in a measurable parameter of disease, and preferably at least 20%, 30%, 40%, 50% or more can be indicative of effective treatment, e.g., normalization of a clinical laboratory value. Efficacy for a given iRNA drug or formulation of that drug can also be judged using an experimental animal model for the given disease as known in the art. When using an experimental animal model, efficacy of treatment is evidenced when a statistically significant reduction in a marker or symptom is observed.

Alternatively, the efficacy can be measured by a reduction in the severity of disease as determined by one skilled in the art of diagnosis based on a clinically accepted disease severity grading scale. Any positive change resulting in e.g., lessening of severity of disease measured using the appropriate scale, represents adequate treatment using an iRNA or iRNA formulation as described herein.

Subjects can be administered a therapeutic amount of iRNA, such as about 0.01 mg/kg to about 200 mg/kg.

The iRNA can be administered by intravenous infusion over a period of time, on a regular basis. In certain embodiments, after an initial treatment regimen, the treatments can be administered on a less frequent basis. Administration of the iRNA can reduce XDH levels, e.g., in at least one of a cell, tissue, blood, urine, or other compartment of the patient by at least about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%, or below the level of detection of the assay method used.

Before administration of a full dose of the iRNA, patients can be administered a smaller dose, such as a 5% infusion reaction, and monitored for adverse effects, such as an allergic reaction. In another example, the patient can be monitored for unwanted immunostimulatory effects, such as increased cytokine (e.g., TNF-alpha or INF-alpha) levels.

Alternatively, the iRNA can be administered subcutaneously, i.e., by subcutaneous injection. One or more injections may be used to deliver the desired daily dose of iRNA to a subject. The injections may be repeated over a period of time. The administration may be repeated on a regular basis. In certain embodiments, after an initial treatment regimen, the treatments can be administered on a less frequent basis. A repeat-dose regimen may include administration of a therapeutic amount of iRNA on a regular basis, such as every other day or to once a year. In certain embodiments, the iRNA is administered about once per month to about once per quarter (i.e., about once every three months).

IX. Cell Type XDH Expression

XDH is expressed predominantly in the liver and intestine, However, adipocytes and other extrahepatic tissues express XDH and thus can contribute significant amounts of uric acid in the body. As a result, silencing XDH mRNA in obese patients with high BMI may result in less uric acid lowering than in lean patients. Additionally, silencing XDH mRNA in the liver may be insufficient to lower urate levels given extrahepatic XDH expression. In patients with reduced, but not complete, urinary uric acid excretion, silencing liver XDH mRNA may be insufficient to reduce urate burden from poor renal clearance (defects such as SLC2A9, ABCG2 etc.) Therefore, the efficacy of the iRNA compounds of the invention to prevent or treat an XDH-associated condition, and the amount required for an effective dose, may depend, at least in part, on the amount of fat present in the subject rather than the overall size of the subject. Therefore, in certain embodiments, the compositions and methods of the invention are not for treatment or not for treatment as a single agent in subjects who are obese, i.e., a body mass index (BMI) ≥30.0 wherein BMI is a person's weight in kilograms divided by the square of height in meters. A high BMI can be an indicator of high body fatness.

This invention is further illustrated by the following examples which should not be construed as limiting. The entire contents of all references, patents and published patent applications cited throughout this application, as well as the Sequence Listing, are hereby incorporated herein by reference.

EXAMPLES

Example 1. iRNA Synthesis

Source of Reagents

Where the source of a reagent is not specifically given herein, such reagent can be obtained from any supplier of reagents for molecular biology at a quality/purity standard for application in molecular biology.

Transcripts

siRNA Design

A set of siRNAs targeting the human XDH "xanthine dehydrogenase" gene (human: NCBI refseqID NM_000379; NCBI GeneID: 7498), as well as toxicology-species XDH orthologs (cynomolgus monkey: XM_005576184; mouse: NM_011723; rat, NM_017154) were designed using custom R and Python scripts. The human NM_000379 REFSEQ mRNA, version 3, has a length of 5717 bases. The rationale and method for the set of siRNA designs is as follows: the predicted efficacy for every potential 19mer siRNA from position 80 through position 5717 (the coding region and 3' UTR) was determined with a linear model derived the direct measure of mRNA knockdown from more than 20,000 distinct siRNA designs targeting a large number of vertebrate genes. Subsets of the XDH siRNAs were designed with perfect or near-perfect matches between human, cynomolgus and rhesus monkey. A further subset was designed with perfect or near-perfect matches to mouse and rat XDH orthologs. For each strand of the siRNA, a custom Python script was used in a brute force search to measure the number and positions of mismatches between the siRNA and all potential alignments in the target species transcriptome. Extra weight was given to mismatches in the seed region, defined here as positions 2-9 of the antisense oligonucleotide, as well the cleavage site of the siRNA, defined here as positions 10-11 of the antisense oligonucleotide. The relative weight of the mismatches was 2.8; 1.2:1 for seed mismatches, cleavage site, and other positions up through antisense position 19. Mismatches in the first position were ignored. A specificity score was calculated for each strand by summing the value of each weighted mismatch. Preference was given to siRNAs whose antisense score in human and cynomolgus monkey was >=2.0 and predicted efficacy was >=50% knockdown of the XDH transcript.

siRNA Synthesis

XDH siRNA sequences are synthesized at 1 umol scale on Mermade 192 synthesizer (BioAutomation) using the solid support mediated phosphoramidite chemistry. The solid support is controlled pore glass (500° A) loaded with custom GalNAc ligand or universal solid support (AM biochemical). Ancillary synthesis reagents, 2'-F and 2'-O-Methyl RNA and deoxy phosphoramidites are obtained from Thermo-Fisher (Milwaukee, Wis.) and Hongene (China). 2'F, 2'-O-Methyl, RNA, DNA and other modified nucleosides are introduced in the sequences using the corresponding phosphoramidites. Synthesis of 3' GalNAc conjugated single strands is performed on a GalNAc modified CPG support. Custom CPG universal solid support is used for the synthesis of antisense single strands. Coupling time for all phosphoramidites (100 mM in acetonitrile) is 5 min employing 5-Ethylthio-1H-tetrazole (ETT) as activator (0.6 M in acetonitrile). Phosphorothioate linkages are generated using a 50 mM solution of 3-((Dimethylamino-methylidene) amino)-3H-1, 2, 4-dithiazole-3-thione (DDTT, obtained from Chemgenes (Wilmington, Mass., USA) in anhydrous acetonitrile/pyridine (1:1 v/v). Oxidation time is 3 minutes. All sequences are synthesized with final removal of the DMT group ("DMT off").

Upon completion of the solid phase synthesis, oligoribonucleotides are cleaved from the solid support and deprotected in sealed 96 deep well plates using 200 μL Aqueous Methylamine reagent at 60° C. for 20 minutes. For sequences containing 2' ribo residues (2'-OH) that are protected with tert-butyl dimethyl silyl (TBDMS) group, a second step deprotection is performed using TEA.3HF (triethylamine trihydro fluoride) reagent. To the methylamine deprotection solution, 200 uL of dimethyl sulfoxide (DMSO) and 300 ul TEA.3HF reagent is added and the solution was incubated for additional 20 min at 60° C. At the end of cleavage and deprotection step, the synthesis plate is allowed to come to room temperature and is precipitated by addition of 1 mL of acetontile: ethanol mixture (9:1). The plates are cooled at −80° C. for 2 hrs and the superanatant decanted carefully with the aid of a multi channel pipette. The oligonucleotide pellet is re-suspended in 20 mM NaOAc buffer and desalted using a 5 mL HiTrap size exclusion column (GE Healthcare) on an AKTA Purifier System equipped with an A905 autosampler and a Frac 950 fraction collector. Desalted samples are collected in 96 well plates. Samples from each sequence are analyzed by LC-MS to confirm the identity, UV (260 nm) for quantification and a selected set of samples by IEX chromatography to determine purity.

Annealing of XDH single strands is performed on a Tecan liquid handling robot. Equimolar mixture of sense and antisense single strands are combined and annealed in 96 well plates. After combining the complementary single strands, the 96 well plate is sealed tightly and heated in an oven at 100° C. for 10 minutes and allowed to come slowly to room temperature over a period 2-3 hours. The concentration of each duplex is normalized to 10 uM in 1×PBS and then submitted for in vitro screening assays.

A detailed list of the unmodified XDH sense and antisense strand sequences is shown in Table 3 and a detailed list of the modified XDH sense and antisense strand sequences is shown in Table 4.

Example 2—In Vitro Screening—Primary Mouse Hepatocytes and Primary Cyno Hepatocytes

Cell Culture and Transfections:

Primary Mouse Hepatocytes (PMH) (GIBCO) and Primary Cyno Hepatocytes (PCH) (Celsis) were transfected by adding 4.9 μl of Opti-MEM plus 0.1 μl of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad Calif. cat #13778-150) to 5 μl of siRNA duplexes per well into a 384-well plate and incubated at room temperature for 15 minutes. Forty μl of William's E Medium (Life Tech) containing about 5×10^3 cells were then added to the siRNA mixture. Cells were incubated for 24 hours prior to RNA purification. Single dose experiments were performed at 10 nM and 0.1 nM.

Total RNA Isolation Using DYNABEADS mRNA Isolation Kit:

RNA was isolated using an automated protocol on a BioTek-EL406 platform using DYNABEADs (Invitrogen, cat #61012). Briefly, 50 μl of Lysis/Binding Buffer and 25 μl of lysis buffer containing 3 μl of magnetic beads were added to the plate with cells. Plates were incubated on an electromagnetic shaker for 10 minutes at room temperature and then magnetic beads were captured and the supernatant was removed. Bead-bound RNA was then washed 2 times with 150 μl Wash Buffer A and once with Wash Buffer B. Beads were then washed with 150 μl Elution Buffer, re-captured and the supernatant was removed.

cDNA Synthesis Using ABI High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, Calif., Cat #4368813):

Ten μl of a master mix containing 1 μl 10× Buffer, 0.4 μl 25× dNTPs, 1 μl 10× Random primers, 0.5 μl Reverse Transcriptase, 0.5 μl RNase inhibitor and 6.6 μl of H_2O per reaction was added to RNA isolated above. Plates were sealed, mixed, and incubated on an electromagnetic shaker

for 10 minutes at room temperature, followed by 2 hours at 37° C. Plates are then incubated at 81° C. for 8 minutes.

Real Time PCR:

Two μl of cDNA were added to a master mix containing 0.5 μl of GAPDH TaqMan Probe (Hs99999905 m1), 0.5 μl XDH probe and 5 μl Lightcycler 480 probe master mix (Roche Cat #04887301001) per well in a 384 well plates (Roche cat #04887301001). Real time PCR was performed in a LightCycler480 Real Time PCR system (Roche). Each duplex was assayed at least two times and data were normalized to cells transfected with a non-targeting control siRNA.

To calculate relative fold change, real time data was analyzed using the ΔΔCt method and normalized to assays performed with cells transfected with 10 nM AD-1955, a non-targeting control siRNA.

The sense and antisense sequences of AD-1955 are:

SENSE:

(SEQ ID NO: 17)
cuuAcGcuGAGuAcuucGAdTsdT;

ANTISENSE:

(SEQ ID NO: 18)
UCGAAGuACUcAGCGuAAGdTsdT.

Table 5 shows the results of a single dose screen in Primary Cynomolgus Hepatocytes and Primary Mouse Hepatocytes transfected with the indicated modified iRNAs.

TABLE 2

Abbreviations of nucleotide monomers used in nucleic acid sequence representation. It will be understood that these monomers, when present in an oligonucleotide, are mutually linked by 5'-3'- phosphodiester bonds.

Abbreviation	Nucleotide(s)
A	Adenosine-3'-phosphate
Af	2'-fluoroadenosine-3'-phosphate
Afs	2'-fluoroadenosine-3'-phosphorothioate
As	adenosine-3'-phosphorothioate
C	cytidine-3'-phosphate
Cf	2'-fluorocytidine-3'-phosphate
Cfs	2'-fluorocytidine-3'-phosphorothioate
Cs	cytidine-3'-phosphorothioate
G	guanosine-3'-phosphate
Gf	2'-fluoroguanosine-3'-phosphate
Gfs	2'-fluoroguanosine-3'-phosphorothioate
Gs	guanosine-3'-phosphorothioate
T	5'-methyluridine-3'-phosphate
Tf	2'-fluoro-5-methyluridine-3'-phosphate
Tfs	2'-fluoro-5-methyluridine-3'-phosphorothioate
Ts	5-methyluridine-3'-phosphorothioate
U	Uridine-3'-phosphate
Uf	2'-fluorouridine-3'-phosphate
Ufs	2'-fluorouridine-3'-phosphorothioate
Us	uridine-3'-phosphorothioate
N	any nucleotide (G, A, C, T or U)
a	2'-O-methyladenosine-3'-phosphate
as	2'-O-methyladenosine-3'-phosphorothioate
c	2'-O-methylcytidine-3'-phosphate
cs	2'-O-methylcytidine-3'-phosphorothioate
g	2'-O-methylguanosine-3'-phosphate
gs	2'-O-methylguanosine-3'-phosphorothioate
t	2'-O-methyl-5-methyluridine-3'-phosphate
ts	2'-O-methyl-5-methyluridine-3'-phosphorothioate
u	2'-O-methyluridine-3'-phosphate
us	2'-O-methyluridine-3'-phosphorothioate
s	phosphorothioate linkage
L96	N-[tris(GalNAc-alkyl)-amidodecanoyl)]-4-hydroxyprolinol Hyp-(GalNAc-alkyl)3
dT	2`-deoxythymidine-3`-phosphate
dC	2`-deoxycytidine-3`-phosphate
Y44	inverted abasic DNA(2-hydroxymethyl-tetrahydrofurane-5-phosphate)
(Tgn)	Thymidine-glycol nucleic acid (GNA) S-Isomer
P	Phosphate
VP	Vinyl-phosphate
(Aam)	2`-O-(N-methylacetamide)adenosine-3`-phosphate

TABLE 3

Unmodified Sense and Antisense Strand Sequences of XDH dsRNAs

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO	Position in NM_000379.3	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO	Position in NM_000379.3
AD-70003	A-140499	AACUGUGGAAG GAAUAGGAAA	19	339-358_G21A	A-140500	UUUCCUAUUCCU UCCACAGUUGU	105	337-358_G21A
AD-70049	A-140591	AACUGUGGAAG GAAUAGGAAA	20	339-358_G21A	A-140592	UUUCCUAUUCCU UCCACAGUUGU	106	337-358_G21A
AD-69981	A-140455	GAGGAGAUUGA GAAUGCCUUA	21	490-509_C21A	A-140456	UAAGGCAUUCUC AAUCUCCUCCA	107	488-509_C21A
AD-70027	A-140547	GAGGAGAUUGA GAAUGCCUUA	22	490-509_C21A	A-140548	UAAGGCAUUCUC AAUCUCCUCCA	108	488-509_C21A
AD-69970	A-140433	GAGAUUGAGAA UGCCUUCCAA	23	493-512	A-140434	UUGGAAGGCAUU CUCAAUCUCCU	109	491-512
AD-70016	A-140525	GAGAUUGAGAA UGCCUUCCAA	24	493-512	A-140526	UUGGAAGGCAUU CUCAAUCUCCU	110	491-512
AD-70007	A-140507	GUUCAAGAAUA UGCUGUUUCA	25	888-907_C21A	A-140508	UGAAACAGCAUA UUCUUGAACUU	111	886-907_C21A

TABLE 3-continued

Unmodified Sense and Antisense Strand Sequences of XDH dsRNAs

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO	Position in NM_000379.3	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO	Position in NM_000379.3
AD-70053	A-140599	GUUCAAGAAUA UGCUGUUUCA	26	888-907_C21A	A-140600	UGAAACAGCAUA UUCUUGAACUU	112	886-907_C21A
AD-69995	A-140483	AAAAGACAGAG GUGUUCAGAA	27	1046-1065_G21A	A-140484	UUCUGAACACCU CUGUCUUUUGG	113	1044-1065_G21A
AD-70041	A-140575	AAAAGACAGAG GUGUUCAGAA	28	1046-1065_G21A	A-140576	UUCUGAACACCU CUGUCUUUUGG	114	1044-1065_G21A
AD-69973	A-140439	AAAGACAGAGG UGUUCAGAGA	29	1047-1066_G21A	A-140440	UCUCUGAACACC UCUGUCUUUUG	115	1045-1066_G21A
AD-70019	A-140531	AAAGACAGAGG UGUUCAGAGA	30	1047-1066_G21A	A-140532	UCUCUGAACACC UCUGUCUUUUG	116	1045-1066_G21A
AD-69975	A-140443	CGGAGAGAAGA UGACAUUGCA	31	1357-1376_C21A	A-140444	UGCAAUGUCAUC UUCUCUCCGGG	117	1355-1376_C21A
AD-70021	A-140535	CGGAGAGAAGA UGACAUUGCA	32	1357-1376_C21A	A-140536	UGCAAUGUCAUC UUCUCUCCGGG	118	1355-1376_C21A
AD-69983	A-140459	AGAGAAGAUGA CAUUGCCAAA	33	1360-1379_G21A	A-140460	UUUGGCAAUGUC AUCUUCUCUCC	119	1358-1379_G21A
AD-70029	A-140551	AGAGAAGAUGA CAUUGCCAAA	34	1360-1379_G21A	A-140552	UUUGGCAAUGUC AUCUUCUCUCC	120	1358-1379_G21A
AD-69986	A-140465	GAAGAUGACAU UGCCAAGGUA	35	1363-1382	A-140466	UACCUUGGCAAU GUCAUCUUCUC	121	1361-1382
AD-70032	A-140557	GAAGAUGACAU UGCCAAGGUA	36	1363-1382	A-140558	UACCUUGGCAAU GUCAUCUUCUC	122	1361-1382
AD-69976	A-140445	CAGUCUGAGGA GGACAUGGUA	37	1783-1802_G21A	A-140446	UACCAUGUCCUC CUCAGACUGAC	123	1781-1802_G21A
AD-70022	A-140537	CAGUCUGAGGA GGACAUGGUA	38	1783-1802_G21A	A-140538	UACCAUGUCCUC CUCAGACUGAC	124	1781-1802_G21A
AD-69987	A-140467	AAGGAUAAGGU UACUUGUGUU	39	2053-2072	A-140468	AACACAAGUAAC CUUAUCCUUCG	125	2051-2072
AD-70033	A-140559	AAGGAUAAGGU UACUUGUGUU	40	2053-2072	A-140560	AACACAAGUAAC CUUAUCCUUCG	126	2051-2072
AD-69990	A-140473	AGGAUAAGGUU ACUUGUGUUA	41	2054-2073_G21A	A-140474	UAACACAAGUAA CCUUAUCCUUC	127	2052-2073_G21A
AD-70036	A-140565	AGGAUAAGGUU ACUUGUGUUA	42	2054-2073_G21A	A-140566	UAACACAAGUAA CCUUAUCCUUC	128	2052-2073_G21A
AD-69989	A-140471	CAUAUCAUUGG UGCUGUGGUU	43	2077-2096	A-140472	AACCACAGCACC AAUGAUAUGCC	129	2075-2096
AD-70035	A-140563	CAUAUCAUUGG UGCUGUGGUU	44	2077-2096	A-140564	AACCACAGCACC AAUGAUAUGCC	130	2075-2096
AD-69988	A-140469	GUGAAAAUCAC CUAUGAAGAA	45	2137-2156	A-140470	UUCUUCAUAGGU GAUUUUCACCC	131	2135-2156
AD-70034	A-140561	GUGAAAAUCAC CUAUGAAGAA	46	2137-2156	A-140562	UUCUUCAUAGGU GAUUUUCACCC	132	2135-2156
AD-69996	A-140485	ACAAUUGAGGA UGCUAUAAAA	47	2173-2192_G21A	A-140486	UUUUAUAGCAUC CUCAAUUGUGA	133	2171-2192_G21A
AD-70042	A-140577	ACAAUUGAGGA UGCUAUAAAA	48	2173-2192_G21A	A-140578	UUUUAUAGCAUC CUCAAUUGUGA	134	2171-2192_G21A
AD-69977	A-140447	UGCUAUAAAGA ACAACUCCUU	49	2184-2203	A-140448	AAGGAGUUGUUC UUUAUAGCAUC	135	2182-2203

TABLE 3-continued

Unmodified Sense and Antisense Strand Sequences of XDH dsRNAs

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO	Position in NM_000379.3	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO	Position in NM_000379.3
AD-70023	A-140539	UGCUAUAAAGA ACAACUCCUU	50	2184-2203	A-140540	AAGGAGUUGUUC UUUAUAGCAUC	136	2182-2203
AD-69978	A-140449	GCUAUAAAGAA CAACUCCUUU	51	2185-2204	A-140450	AAAGGAGUUGUU CUUUAUAGCAU	137	2183-2204
AD-70024	A-140541	GCUAUAAAGAA CAACUCCUUU	52	2185-2204	A-140542	AAAGGAGUUGUU CUUUAUAGCAU	138	2183-2204
AD-69972	A-140437	UAUAAAGAACA ACUCCUUUUA	53	2187-2206	A-140438	UAAAAGGAGUUG UUCUUUAUAGC	139	2185-2206
AD-70018	A-140529	UAUAAAGAACA ACUCCUUUUA	54	2187-2206	A-140530	UAAAAGGAGUUG UUCUUUAUAGC	140	2185-2206
AD-69971	A-140435	UAAAGAACAAC UCCUUUUAUA	55	2189-2208_G21A	A-140436	UAUAAAAGGAGU UGUUCUUUAUA	141	2187-2208_G21A
AD-70017	A-140527	UAAAGAACAAC UCCUUUUAUA	56	2189-2208_G21A	A-140528	UAUAAAAGGAGU UGUUCUUUAUA	142	2187-2208_G21A
AD-69994	A-140481	GACAUGCUGAU AACUGGUGGA	57	2578-2597_C21A	A-140482	UCCACCAGUUAU CAGCAUGUCCU	143	2576-2597_C21A
AD-70040	A-140573	GACAUGCUGAU AACUGGUGGA	58	2578-2597_C21A	A-140574	UCCACCAGUUAU CAGCAUGUCCU	144	2576-2597_C21A
AD-69985	A-140463	AUGCUGAUAAC UGGUGGCAGA	59	2581-2600	A-140464	UCUGCCACCAGU UAUCAGCAUGU	145	2579-2600
AD-70031	A-140555	AUGCUGAUAAC UGGUGGCAGA	60	2581-2600	A-140556	UCUGCCACCAGU UAUCAGCAUGU	146	2579-2600
AD-69982	A-140457	UGAUAACUGGU GGCAGACAUA	61	2585-2604_C21A	A-140458	UAUGUCUGCCAC CAGUUAUCAGC	147	2583-2604_C21A
AD-70028	A-140549	UGAUAACUGGU GGCAGACAUA	62	2585-2604_C21A	A-140550	UAUGUCUGCCAC CAGUUAUCAGC	148	2583-2604_C21A
AD-69984	A-140461	UACAAGGUUGG CUUCAUGAAA	63	2620-2639_G21A	A-140462	UUUCAUGAAGCC AACCUUGUAUC	149	2618-2639_G21A
AD-70030	A-140553	UACAAGGUUGG CUUCAUGAAA	64	2620-2639_G21A	A-140554	UUUCAUGAAGCC AACCUUGUAUC	150	2618-2639_G21A
AD-69991	A-140475	ACAAGGUUGGC UUCAUGAAGA	65	2621-2640	A-140476	UCUUCAUGAAGC CAACCUUGUAU	151	2619-2640
AD-70037	A-140567	ACAAGGUUGGC UUCAUGAAGA	66	2621-2640	A-140568	UCUUCAUGAAGC CAACCUUGUAU	152	2619-2640
AD-70005	A-140503	AAGCUUGAGGG UUUCACCUUA	67	2956-2975_G21A	A-140504	UAAGGUGAAACC CUCAAGCUUCU	153	2954-2975_G21A
AD-70051	A-140595	AAGCUUGAGGG UUUCACCUUA	68	2956-2975_G21A	A-140596	UAAGGUGAAACC CUCAAGCUUCU	154	2954-2975_G21A
AD-70002	A-140497	AAGAGUGAGGU UGACAAGUUA	69	3025-3044_C21A	A-140498	UAACUUGUCAAC CUCACUCUUCC	155	3023-3044_C21A
AD-70048	A-140589	AAGAGUGAGGU UGACAAGUUA	70	3025-3044_C21A	A-140590	UAACUUGUCAAC CUCACUCUUCC	156	3023-3044_C21A
AD-69992	A-140477	GUUCAACAAGG AGAAUUGUUA	71	3042-3061_G21A	A-140478	UAACAAUUCUCC UUGUUGAACUU	157	3040-3061_G21A
AD-70038	A-140569	GUUCAACAAGG AGAAUUGUUA	72	3042-3061_G21A	A-140570	UAACAAUUCUCC UUGUUGAACUU	158	3040-3061_G21A
AD-69993	A-140479	ACAAGGAGAAU UGUUGGAAAA	73	3047-3066	A-140480	UUUUCCAACAAU UCUCCUUGUUG	159	3045-3066

TABLE 3-continued

Unmodified Sense and Antisense Strand Sequences of XDH dsRNAs

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO	Position in NM_000379.3	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO	Position in NM_000379.3
AD-70039	A-140571	ACAAGGAGAAU UGUUGGAAAA	74	3047-3066	A-140572	UUUUCCAACAAU UCUCCUUGUUG	160	3045-3066
AD-69980	A-140453	ACCAAGUUUGG AAUAAGCUUU	75	3091-3110	A-140454	AAAGCUUAUUCC AAACUUGGUGG	161	3089-3110
AD-70026	A-140545	ACCAAGUUUGG AAUAAGCUUU	76	3091-3110	A-140546	AAAGCUUAUUCC AAACUUGGUGG	162	3089-3110
AD-69979	A-140451	UAUCUUCUUUG CCAUCAAAGA	77	3891-3910	A-140452	UCUUUGAUGGCA AAGAAGAUAGA	163	3889-3910
AD-70025	A-140543	UAUCUUCUUUG CCAUCAAAGA	78	3891-3910	A-140544	UCUUUGAUGGCA AAGAAGAUAGA	164	3889-3910
AD-69974	A-140441	UCUUCUUUGCC AUCAAAGAUA	79	3893-3912_G21A	A-140442	UAUCUUUGAUGG CAAAGAAGAUA	165	3891-3912_G21A
AD-70020	A-140533	UCUUCUUUGCC AUCAAAGAUA	80	3893-3912_G21A	A-140534	UAUCUUUGAUGG CAAAGAAGAUA	166	3891-3912_G21A
AD-70004	A-140501	CAGAACAUGGA UCUAUUAAAA	81	4152-4171_G21A	A-140502	UUUUAAUAGAUC CAUGUUCUGUG	167	4150-4171_G21A
AD-70050	A-140593	CAGAACAUGGA UCUAUUAAAA	82	4152-4171_G21A	A-140594	UUUUAAUAGAUC CAUGUUCUGUG	168	4150-4171_G21A
AD-70009	A-140511	ACAAUGAUAAG CAAAUUCAAA	83	4266-4285	A-140512	UUUGAAUUUGCU UAUCAUUGUGU	169	4264-4285
AD-70055	A-140603	ACAAUGAUAAG CAAAUUCAAA	84	4266-4285	A-140604	UUUGAAUUUGCU UAUCAUUGUGU	170	4264-4285
AD-69998	A-140489	AAUGGUGAAUA UGCAAUUAGA	85	4300-4319_G21A	A-140490	UCUAAUUGCAUA UUCACCAUUUA	171	4298-4319_G21A
AD-70044	A-140581	AAUGGUGAAUA UGCAAUUAGA	86	4300-4319_G21A	A-140582	UCUAAUUGCAUA UUCACCAUUUA	172	4298-4319_G21A
AD-70006	A-140505	ACCAAUGAACA GCAAAGCAUA	87	4519-4538	A-140506	UAUGCUUUGCUG UUCAUUGGUUU	173	4517-4538
AD-70052	A-140597	ACCAAUGAACA GCAAAGCAUA	88	4519-4538	A-140598	UAUGCUUUGCUG UUCAUUGGUUU	174	4517-4538
AD-70001	A-140495	CCAUCUUUGAA UCAUUGGAAA	89	4599-4618	A-140496	UUUCCAAUGAUU CAAAGAUGGUU	175	4597-4618
AD-70047	A-140587	CCAUCUUUGAA UCAUUGGAAA	90	4599-4618	A-140588	UUUCCAAUGAUU CAAAGAUGGUU	176	4597-4618
AD-69997	A-140487	AAGAAUAAAGA AUGAAACAAA	91	4618-4637	A-140488	UUUGUUUCAUUC UUUAUUCUUUC	177	4616-4637
AD-70043	A-140579	AAGAAUAAAGA AUGAAACAAA	92	4618-4637	A-140580	UUUGUUUCAUUC UUUAUUCUUUC	178	4616-4637
AD-69999	A-140491	AAUAAAGAAUG AAACAAAUUA	93	4621-4640_C21A	A-140492	UAAUUUGUUUCA UUCUUUAUUCU	179	4619-4640_C21A
AD-70045	A-140583	AAUAAAGAAUG AAACAAAUUA	94	4621-4640_C21A	A-140584	UAAUUUGUUUCA UUCUUUAUUCU	180	4619-4640_C21A
AD-70008	A-140509	AUCCAACCAAC UCAAUUAUUA	95	4703-4722_G21A	A-140510	UAAUAAUUGAGU UGGUUGGAUUU	181	4701-4722_G21A
AD-70054	A-140601	AUCCAACCAAC UCAAUUAUUA	96	4703-4722_G21A	A-140602	UAAUAAUUGAGU UGGUUGGAUUU	182	4701-4722_G21A
AD-70000	A-140493	CACUGUAUAAA UCCAACCUUA	97	5599-5618_C21A	A-140494	UAAGGUUGGAUU UAUACAGUGAA	183	5597-5618_C21A

TABLE 3-continued

Unmodified Sense and Antisense Strand Sequences of XDH dsRNAs

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO	Position in NM_000379.3	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO	Position in NM_000379.3
AD-70046	A-140585	CACUGUAUAAA UCCAACCUUA	98	5599-5618_C21A	A-140586	UAAGGUUGGAUU UAUACAGUGAA	184	5597-5618_C21A
AD-70015	A-140523	AACUGUGGAAG GCAUAGGAAA	99	337-356	A-140524	UUUCCUAUGCCU UCCACAGUUGU	185	335-356
AD-70012	A-140517	GGAUUUCAAAC CUUUAGAUCA	100	673-692_C21A	A-140518	UGAUCUAAAGGU UUGAAAUCCUC	186	671-692_C21A
AD-70014	A-140521	ACAGAGAUAGG CAUUGAAAUA	101	860-879_G21A	A-140522	UAUUUCAAUUCC UAUCUCUGUGU	187	858-879_G21A
AD-70010	A-140513	GGCAUUGAAAU GAAAUUUAAA	102	869-888	A-140514	UUUAAAUUUCAU UUCAAUGCCUA	188	867-888
AD-70013	A-140519	ACAAUCCAGGA UGCUAUAAAA	103	2168-2187_G21A	A-140520	UUUUAUAGCAUC CUGGAUUGUGA	189	2166-2187_G21A
AD-70011	A-140515	CAAGAUGGAAG UGGAGAAAUU	104	3019-3038	A-140516	AAUUUCUCCACU UCCAUCUUGCG	190	3017-3038

TABLE 4

XDH Modified Sequences

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO	Start in NM_000379.3	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO	Start in NM_000379.3
AD-70003	A-140499	AACUGUGGAAGGAAU AGGAAAdTdT	191	339	A-140500	UUUCCUAUUCCUUCCAC AGUUGUdTdT	277	337
AD-70049	A-140591	asascuguGfgAfAf GfgaauaggaaaL96	192	339	A-140592	usUfsuccUfaUfUfcc uuCfcAfcaguusgsu	278	337
AD-69981	A-140455	GAGGAGAUUGAGAAU GCCUUAdTdT	193	490	A-140456	UAAGGCAUUCUCAAUCU CCUCCAdTdT	279	488
AD-70027	A-140547	gsasggagAfuUfGf AfgaaugccuuaL96	194	490	A-140548	usAfsaggCfaUfUfcu caAfuCfuccucscsa	280	488
AD-69970	A-140433	GAGAUUGAGAAUGCC UUCCAAdTdT	195	493	A-140434	UUGGAAGGCAUUCUCAA UCUCCUdTdT	281	491
AD-70016	A-140525	gsasgauuGfaGfAf AfugccuuccaaL96	196	493	A-140526	usUfsggaAfgGfCfau ucUfcAfaucucscsu	282	491
AD-70007	A-140507	GUUCAAGAAUAUGCU GUUUCAdTdT	197	888	A-140508	UGAAACAGCAUAUUCUU GAACUUdTdT	283	886
AD-70053	A-140599	gsusucaaGfaAfUf AfugcuguuucaL96	198	888	A-140600	usGfsaaaCfaGfCfau auUfcUfugaacsusu	284	886
AD-69995	A-140483	AAAAGACAGAGGUGU UCAGAAdTdT	199	1046	A-140484	UUCUGAACACCUCUGUC UUUUGGdTdT	285	1044
AD-70041	A-140575	asasaagaCfaGfAf GfguguucagaaL96	200	1046	A-140576	usUfscugAfaCfAfcc ucUfgUfcuuuusgsg	286	1044
AD-69973	A-140439	AAAGACAGAGGUGUU CAGAGAdTdT	201	1047	A-140440	UCUCUGAACACCUCUGU CUUUUGdTdT	287	1045
AD-70019	A-140531	asasagacAfgAfGf GfuguucagagaL96	202	1047	A-140532	usCfsucuGfaAfCfac cuCfuGfucuuususg	288	1045
AD-69975	A-140443	CGGAGAGAAGAUGAC AUUGCAdTdT	203	1357	A-140444	UGCAAUGUCAUCUUCUC UCCGGGdTdT	289	1355
AD-70021	A-140535	csgsgagaGfaAfGf AfugacauugcaL96	204	1357	A-140536	usGfscaaUfgUfCfau cuUfcUfcuccgsgsg	290	1355

TABLE 4-continued

XDH Modified Sequences

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO	Start in NM_ 000379.3	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO	Start in NM_ 000379.3
AD-69983	A-140459	AGAGAAGAUGACAUU GCCAAAdTdT	205	1360	A-140460	UUUGGCAAUGUCAUCUU CUCUCCdTdT	291	1358
AD-70029	A-140551	asgsagaaGfaUfGf AfcauugccaaaL96	206	1360	A-140552	usUfsuggCfaAfUfgu caUfcUfucucuscsc	292	1358
AD-69986	A-140465	GAAGAUGACAUUGCC AAGGUAdTdT	207	1363	A-140466	UACCUUGGCAAUGUCAU CUUCUCdTdT	293	1361
AD-70032	A-140557	gsasagauGfaCfAf Ufugccaaggual96	208	1363	A-140558	usAfsccuUfgGfCfaa ugUfcAfucuucsusc	294	1361
AD-69976	A-140445	CAGUCUGAGGAGGAC AUGGUAdTdT	209	1783	A-140446	UACCAUGUCCUCCUCAG ACUGACdTdT	295	1781
AD-70022	A-140537	csasgucuGfaGfGf Afggacauggual96	210	1783	A-140538	usAfsccaUfgUfCfcu ccUfcAfgacugsasc	296	1781
AD-69987	A-140467	AAGGAUAAGGUUACU UGUGUUdTdT	211	2053	A-140468	AACACAAGUAACCUUAU CCUUCGdTdT	297	2051
AD-70033	A-140559	asasggauAfaGfGf Ufuacuuguguul96	212	2053	A-140560	asAfscacAfaGfUfaa ccUfuAfuccuuscsg	298	2051
AD-69990	A-140473	AGGAUAAGGUUACUU GUGUUAdTdT	213	2054	A-140474	UAACACAAGUAACCUUA UCCUUCdTdT	299	2052
AD-70036	A-140565	asgsgauaAfgGfUf UfacuuguguuaL96	214	2054	A-140566	usAfsacaCfaAfGfua acCfuUfauccususc	300	2052
AD-69989	A-140471	CAUAUCAUUGGUGCU GUGGUUdTdT	215	2077	A-140472	AACCACAGCACCAAUGA UAUGCCdTdT	301	2075
AD-70035	A-140563	csasuaucAfuUfGf GfugcugugguuL96	216	2077	A-140564	asAfsccaCfaGfCfac caAfuGfauaugscsc	302	2075
AD-69988	A-140469	GUGAAAAUCACCUAU GAAGAAdTdT	217	2137	A-140470	UUCUUCAUAGGUGAUUU UCACCCdTdT	303	2135
AD-70034	A-140561	gsusgaaaAfuCfAf CfcuaugaagaaL96	218	2137	A-140562	usUfscuuCfaUfAfgg ugAfuUfuucacscsc	304	2135
AD-69996	A-140485	ACAAUUGAGGAUGCU AUAAAAdTdT	219	2173	A-140486	UUUUAUAGCAUCCUCAA UUGUGAdTdT	305	2171
AD-70042	A-140577	ascsaauuGfaGfGf AfugcuauaaaaL96	220	2173	A-140578	usUfsuuaUfaGfCfau ccUfcAfauugusgsa	306	2171
AD-69977	A-140447	UGCUAUAAAGAACAA CUCCUUdTdT	221	2184	A-140448	AAGGAGUUGUUCUUUAU AGCAUCdTdT	307	2182
AD-70023	A-140539	usgscuauAfaAfGf AfacaacuccuuL96	222	2184	A-140540	asAfsggaGfuUfGfuu cuUfuAfuagcasusc	308	2182
AD-69978	A-140449	GCUAUAAAGAACAAC UCCUUUdTdT	223	2185	A-140450	AAAGGAGUUGUUCUUUA UAGCAUdTdT	309	2183
AD-70024	A-140541	gscsuauaAfaGfAf AfcaacuccuuuL96	224	2185	A-140542	asAfsaggAfgUfUfgu ucUfuUfauagcsasu	310	2183
AD-69972	A-140437	UAUAAAGAACAACUC CUUUUAdTdT	225	2187	A-140438	UAAAAGGAGUUGUUCUU UAUAGCdTdT	311	2185
AD-70018	A-140529	usasuaaaGfaAfCf AfacuccuuuuaL96	226	2187	A-140530	usAfsaaaGfgAfGfuu guUfcUfuuauasgsc	312	2185
AD-69971	A-140435	UAAAGAACAACUCCU UUUAUAdTdT	227	2189	A-140436	UAUAAAAGGAGUUGUUC UUUAUAdTdT	313	2187
AD-70017	A-140527	usasaagaAfcAfAf CfuccuuuuauaL96	228	2189	A-140528	usAfsuaaAfaGfGfag uuGfuUfcuuuasusa	314	2187

TABLE 4-continued

XDH Modified Sequences

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO	Start in NM_ 000379.3	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO	Start in NM_ 000379.3
AD-69994	A-140481	GACAUGCUGAUAACU GGUGGAdTdT	229	2578	A-140482	UCCACCAGUUAUCAGCA UGUCCUdTdT	315	2576
AD-70040	A-140573	gsascaugCfuGfAf UfaacugguggaL96	230	2578	A-140574	usCfscacCfaGfUfua ucAfgCfaugucscsu	316	2576
AD-69985	A-140463	AUGCUGAUAACUGGU GGCAGAdTdT	231	2581	A-140464	UCUGCCACCAGUUAUCA GCAUGUdTdT	317	2579
AD-70031	A-140555	asusgcugAfuAfAf CfugguggcagaL96	232	2581	A-140556	usCfsugcCfaCfCfag uuAfuCfagcausgsu	318	2579
AD-69982	A-140457	UGAUAACUGGUGGCA GACAUAdTdT	233	2585	A-140458	UAUGUCUGCCACCAGUU AUCAGCdTdT	319	2583
AD-70028	A-140549	usgsauaaCfuGfGf Ufggcagacaual96	234	2585	A-140550	usAfsuguCfuGfCfca ccAfgUfuaucasgsc	320	2583
AD-69984	A-140461	UACAAGGUUGGCUUC AUGAAAdTdT	235	2620	A-140462	UUUCAUGAAGCCAACCU UGUAUCdTdT	321	2618
AD-70030	A-140553	usascaagGfuUfGf Gfcuucaugaaal96	236	2620	A-140554	usUfsucaUfgAfAfgc caAfcCfuuguasusc	322	2618
AD-69991	A-140475	ACAAGGUUGGCUUCA UGAAGAdTdT	237	2621	A-140476	UCUUCAUGAAGCCAACC UUGUAUdTdT	323	2619
AD-70037	A-140567	ascsaaggUfuGfGf Cfuucaugaagal96	238	2621	A-140568	usCfsuucAfuGfAfag ccAfaCfcuugusasu	324	2619
AD-70005	A-140503	AAGCUUGAGGGUUUC ACCUUAdTdT	239	2956	A-140504	UAAGGUGAAACCCUCAA GCUUCUdTdT	325	2954
AD-70051	A-140595	asasgcuuGfaGfGf Gfuuucaccuual96	240	2956	A-140596	usAfsaggUfgAfAfac ccUfcAfagcuuscsu	326	2954
AD-70002	A-140497	AAGAGUGAGGUUGAC AAGUUAdTdT	241	3025	A-140498	UAACUUGUCAACCUCAC UCUUCCdTdT	327	3023
AD-70048	A-140589	asasgaguGfaGfGf Ufugacaaguual96	242	3025	A-140590	usAfsacuUfgUfCfaa ccUfcAfcucuuscsc	328	3023
AD-69992	A-140477	GUUCAACAAGGAGAA UUGUUAdTdT	243	3042	A-140478	UAACAAUUCUCCUUGUU GAACUUdTdT	329	3040
AD-70038	A-140569	gsusucaaCfaAfGf Gfagaauuguual96	244	3042	A-140570	usAfsacaAfuUfCfuc cuUfgUfugaacsusu	330	3040
AD-69993	A-140479	ACAAGGAGAAUUGUU GGAAAAdTdT	245	3047	A-140480	UUUUCCAACAAUUCUCC UUGUUGdTdT	331	3045
AD-70039	A-140571	ascsaaggAfgAfAf Ufuguuggaaaal96	246	3047	A-140572	usUfsuucCfaAfCfaa uuCfuCfcuugususg	332	3045
AD-69980	A-140453	ACCAAGUUUGGAAUA AGCUUUdTdT	247	3091	A-140454	AAAGCUUAUUCCAAACU UGGUGGdTdT	333	3089
AD-70026	A-140545	ascscaagUfuUfGf Gfaauaagcuuul96	248	3091	A-140546	asAfsagcUfuAfUfuc caAfaCfuuggusgsg	334	3089
AD-69979	A-140451	UAUCUUCUUUGCCAU CAAAGAdTdT	249	3891	A-140452	UCUUUGAUGGCAAAGAA GAUAGAdTdT	335	3889
AD-70025	A-140543	usasucuuCfuUfUf Gfccaucaaagal96	250	3891	A-140544	usCfsuuuGfaUfGfgc aaAfgAfagauasgsa	336	3889
AD-69974	A-140441	UCUUCUUUGCCAUCA AAGAUAdTdT	251	3893	A-140442	UAUCUUUGAUGGCAAAG AAGAUAdTdT	337	3891
AD-70020	A-140533	uscsuucuUfuGfCf CfaucaaagauaL96	252	3893	A-140534	usAfsucuUfuGfAfug gcAfaAfgaagasusa	338	3891

TABLE 4-continued

XDH Modified Sequences

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO	Start in NM_ 000379.3	Antisense Oligo Name	Antisense Sequence (5' to 3')	SEQ ID NO	Start in NM_ 000379.3
AD-70004	A-140501	CAGAACAUGGAUCUA UUAAAAdTdT	253	4152	A-140502	UUUUAAUAGAUCCAUGU UCUGUGdTdT	339	4150
AD-70050	A-140593	csasgaacAfuGfGf Afucuauuaaaa L96	254	4152	A-140594	usUfsuuaAfuAfGfau ccAfuGfuucugsusg	340	4150
AD-70009	A-140511	ACAAUGAUAAGCAAA UUCAAAdTdT	255	4266	A-140512	UUUGAAUUUGCUUAUCA UUGUGUdTdT	341	4264
AD-70055	A-140603	ascsaaugAfuAfAf GfcaaauucaaaL96	256	4266	A-140604	usUfsugaAfuUfUfgc uuAfuCfauugusgsu	342	4264
AD-69998	A-140489	AAUGGUGAAUAUGCA AUUAGAdTdT	257	4300	A-140490	UCUAAUUGCAUAUUCAC CAUUUAdTdT	343	4298
AD-70044	A-140581	asasugguGfaAfUf Afugcaauuaga L96	258	4300	A-140582	usCfsuaaUfuGfCfau auUfcAfccauususa	344	4298
AD-70006	A-140505	ACCAAUGAACAGCAA AGCAUAdTdT	259	4519	A-140506	UAUGCUUUGCUGUUCAU UGGUUUdTdT	345	4517
AD-70052	A-140597	ascscaauGfaAfCf Afgcaaagcaua L96	260	4519	A-140598	usAfsugcUfuUfGfcu guUfcAfuuggususu	346	4517
AD-70001	A-140495	CCAUCUUUGAAUCAU UGGAAAdTdT	261	4599	A-140496	UUUCCAAUGAUUCAAAG AUGGUUdTdT	347	4597
AD-70047	A-140587	cscsaucuUfuGfAf Afucauuggaaa L96	262	4599	A-140588	usUfsuccAfaUfGfau ucAfaAfgauggsusu	348	4597
AD-69997	A-140487	AAGAAUAAAGAAUGA AACAAAdTdT	263	4618	A-140488	UUUGUUUCAUUCUUUAU UCUUUCdTdT	349	4616
AD-70043	A-140579	asasgaauAfaAfGf Afaugaaacaaa L96	264	4618	A-140580	usUfsuguUfuCfAfuu cuUfuAfuucuususc	350	4616
AD-69999	A-140491	AAUAAAGAAUGAAAC AAAUUAdTdT	265	4621	A-140492	UAAUUUGUUUCAUUCUU UAUUCUdTdT	351	4619
AD-70045	A-140583	asasuaaaGfaAfUf Gfaaacaaauua L96	266	4621	A-140584	usAfsauuUfgUfUfuc auUfcUfuuauuscsu	352	4619
AD-70008	A-140509	AUCCAACCAACUCAA UUAUUAdTdT	267	4703	A-140510	UAAUAAUUGAGUUGGUU GGAUUUdTdT	353	4701
AD-70054	A-140601	asusccaaCfcAfAf CfucaauuauuaL96	268	4703	A-140602	usAfsauaAfuUfGfag uuGfgUfuggaususu	354	4701
AD-70000	A-140493	CACUGUAUAAAUCCA ACCUUAdTdT	269	5599	A-140494	UAAGGUUGGAUUUAUAC AGUGAAdTdT	355	5597
AD-70046	A-140585	csascuguAfuAfAf AfuccaaccuuaL96	270	5599	A-140586	usAfsaggUfuGfGfau uuAfuAfcagugsasa	356	5597
AD-70015	A-140523	AACUGUGGAAGGCAU AGGAAAdTdT	271	337	A-140524	UUUCCUAUGCCUUCCAC AGUUGUdTdT	357	335
AD-70012	A-140517	GGAUUUCAAACCUUU AGAUCAdTdT	272	673	A-140518	UGAUCUAAAGGUUUGAA AUCCUCdTdT	358	671
AD-70014	A-140521	ACAGAGAUAGGCAUU GAAAUAdTdT	273	860	A-140522	UAUUUCAAUGCCUAUCU CUGUGUdTdT	359	858
AD-70010	A-140513	GGCAUUGAAAUGAAA UUUAAAdTdT	274	869	A-140514	UUUAAAUUUCAUUUCAA UGCCUAdTdT	360	867
AD-70013	A-140519	ACAAUCCAGGAUGCU AUAAAAdTdT	275	2168	A-140520	UUUUAUAGCAUCCUGGA UUGUGAdTdT	361	2166
AD-70011	A-140515	CAAGAUGGAAGUGGA GAAAUUdTdT	276	3019	A-140516	AAUUUCUCCACUUCCAU CUUGCGdTdT	362	3017

TABLE 5

Single dose screen in Primary Cynomolgus Hepatocytes and Primary Mouse Hepatocytes

	Primary Cynomolgus Hepatocytes				Primary Mouse Hepatocytes			
Duplex ID	10 nM Avg	10 nM SD	0.1 nM Avg	0.1 nM SD	10 nM Avg	10 nM SD	0.1 nM Avg	0.1 nM SD
AD-70016	34.3	6.9	41.7	5.2	4.5	0.6	38.1	3.4
AD-70017	65.5	5.0	66.2	4.2	8.0	2.5	73.6	7.1
AD-70018	36.3	0.9	74.9	18.3	5.9	1.1	49.6	6.7
AD-70019	59.4	9.2	62.9	13.3	19.8	0.5	123.9	18.8
AD-70020	40.5	7.0	38.9	6.6	10.7	0.8	122.7	24.0
AD-70021	41.0	2.1	72.5	7.6	13.7	1.5	88.1	5.8
AD-70022	44.8	5.9	49.7	19.0	17.3	2.3	105.7	7.6
AD-70023	37.2	9.0	71.7	11.0	5.6	1.7	30.6	5.9
AD-70024	61.0	10.5	86.4	9.3	7.2	0.6	32.6	4.0
AD-70025	40.9	9.2	80.1	9.1	35.3	3.8	109.3	4.9
AD-70026	41.1	5.7	54.1	8.1	7.3	1.1	57.8	4.8
AD-70027	39.0	5.5	57.9	7.2	11.8	1.6	102.4	18.8
AD-70028	47.1	2.6	55.1	15.9	11.0	2.2	95.9	17.2
AD-70029	41.5	7.5	62.7	9.8	8.3	2.0	63.5	11.2
AD-70030	42.4	5.9	45.2	11.0	16.6	1.8	101.1	7.5
AD-70031	52.4	2.2	67.8	20.1	38.0	4.6	99.5	11.6
AD-70032	51.1	6.0	74.3	14.9	22.4	9.9	100.9	12.8
AD-70033	19.6	6.7	45.7	7.5	6.2	0.8	27.2	6.9
AD-70034	39.0	9.1	88.9	22.1	16.2	3.8	89.2	6.2
AD-70035	37.4	16.3	84.0	6.5	16.1	1.7	96.5	7.7
AD-70036	53.0	5.9	61.3	11.3	49.8	7.1	105.9	8.4
AD-70037	46.3	9.1	102.4	23.5	17.1	3.5	97.7	12.5
AD-70038	49.0	3.6	54.3	17.6	86.4	5.3	95.6	11.5
AD-70039	56.3	10.5	85.4	11.4	75.0	5.8	101.6	10.3
AD-70040	52.8	3.5	101.1	17.9	95.9	5.1	96.2	11.1
AD-70041	66.5	20.5	85.7	12.1	48.7	3.0	97.7	5.3
AD-70042	34.8	4.6	47.4	4.2	20.3	1.1	106.8	11.1
AD-70043	42.0	4.7	85.8	22.5	117.6	19.4	113.4	24.1
AD-70044	37.5	9.6	47.1	22.7	87.4	13.6	111.6	16.7
AD-70045	50.9	4.8	84.4	18.3	95.5	5.4	108.8	5.4
AD-70046	81.3	20.3	61.2	21.6	95.5	5.4	111.8	18.5
AD-70047	59.7	12.4	82.7	28.2	100.0	9.7	93.2	14.2
AD-70048	46.7	8.6	61.3	10.3	96.8	11.9	109.7	23.7
AD-70049	33.8	10.8	76.7	15.3	20.4	5.7	95.4	6.8
AD-70050	27.1	4.7	57.7	4.9	107.8	11.0	101.1	5.2
AD-70051	27.6	7.3	76.8	6.7	101.5	14.5	99.1	12.7
AD-70052	36.2	13.1	37.6	14.8	110.3	11.4	109.3	14.8
AD-70053	26.7	5.9	86.9	15.1	104.6	21.4	100.9	9.7
AD-70055	36.5	7.6	58.9	16.7	105.0	14.5	98.7	8.0
AD-69970	47.5	4.5	48.5	7.1	6.1	0.9	22.5	7.7
AD-69971	60.2	11.6	88.4	25.0	10.1	4.8	33.2	10.4
AD-69972	52.8	4.5	67.8	17.0	6.5	1.9	25.5	10.9
AD-69973	36.2	5.3	64.5	13.0	46.6	13.2	50.1	13.9
AD-69974	30.3	3.5	60.8	11.2	9.1	1.5	44.8	11.0
AD-69975	29.7	3.2	48.6	8.2	12.5	6.2	20.3	3.2
AD-69976	40.4	6.0	67.6	5.4	10.4	2.3	45.9	10.3
AD-69977	45.7	7.1	91.6	12.5	6.3	0.9	19.7	3.4
AD-69979	25.8	4.3	58.0	7.1	14.6	9.8	37.8	2.9
AD-69980	30.9	7.9	51.6	13.5	9.5	2.6	23.1	6.3
AD-69981	37.0	6.0	44.2	9.2	14.2	10.8	47.8	3.0
AD-69982	22.6	2.8	48.4	10.7	9.3	1.2	30.4	10.0
AD-69983	31.1	3.6	49.7	10.7	7.9	0.8	27.8	5.6
AD-69984	39.7	6.8	66.2	13.0	49.0	1.1	87.8	13.2
AD-69985	39.2	4.5	47.7	5.4	14.0	3.9	40.0	8.3
AD-69986	21.0	8.3	55.4	7.9	6.8	0.7	24.0	0.7
AD-69987	18.6	6.0	49.4	10.0	11.5	6.0	14.3	3.6
AD-69988	29.5	3.1	56.4	9.9	10.7	3.7	17.2	5.5
AD-69989	28.4	7.8	61.5	8.3	13.3	7.5	22.9	5.7
AD-69990	28.4	4.0	66.5	18.3	13.5	7.9	46.4	8.6
AD-69991	37.4	8.1	48.6	3.8	12.4	1.3	52.4	4.5
AD-69992	31.3	5.6	63.3	23.3	117.7	27.8	101.8	4.3
AD-69993	40.4	3.1	54.4	5.3	86.3	12.9	86.7	9.5
AD-69994	30.1	7.5	60.5	5.5	46.0	15.2	78.7	11.6
AD-69995	51.9	7.6	98.7	22.7	17.0	2.3	72.0	3.2
AD-69996	34.3	4.5	43.2	4.9	72.1	14.5	89.4	9.5
AD-69998	37.3	4.3	64.0	2.8	120.6	15.1	105.5	15.8

TABLE 5-continued

Single dose screen in Primary Cynomolgus Hepatocytes and Primary Mouse Hepatocytes

	Primary Cynomolgus Hepatocytes				Primary Mouse Hepatocytes			
Duplex ID	10 nM Avg	10 nM SD	0.1 nM Avg	0.1 nM SD	10 nM Avg	10 nM SD	0.1 nM Avg	0.1 nM SD
AD-69999	60.4	13.0	97.6	20.1	122.9	40.3	92.6	12.9
AD-70000	74.5	11.1	84.3	9.5	120.4	15.4	97.9	27.4
AD-70001	39.8	5.9	73.5	11.2	121.6	16.8	101.7	19.2
AD-70002	35.7	3.3	63.0	6.9	102.2	8.7	84.2	15.9
AD-70003	26.4	4.3	55.7	5.1	9.8	3.3	43.1	11.1
AD-70004	42.1	5.9	58.1	15.0	130.1	45.5	104.0	9.4
AD-70005	25.7	2.5	53.8	5.4	146.4	47.4	102.7	18.9
AD-70006	31.6	3.7	50.4	1.5	110.2	19.2	106.4	15.4
AD-70007	31.1	4.4	49.5	4.9	120.1	16.3	89.2	3.0
AD-70008	40.2	3.6	64.4	3.2	106.1	13.1	105.6	22.0
AD-70009	39.7	5.4	63.7	3.5	112.1	8.1	98.7	20.3
AD-70010	96.3	26.2	110.6	11.7	5.2	1.4	10.2	3.4
AD-70011	77.0	10.7	102.9	12.6	10.3	1.1	15.9	2.0
AD-70012	92.4	23.9	95.6	7.4	8.1	1.4	16.1	2.7
AD-70013	44.8	9.2	79.9	2.3	6.7	0.8	14.3	3.0
AD-70014	84.6	17.7	88.4	9.5	6.9	1.6	16.2	2.8
AD-70015	48.4	14.9	80.8	6.2	8.0	2.2	14.5	2.4

Example 3. iRNA Synthesis and in Vitro Screening Primary Human Hepatocytes siRNA Design

A set of siRNAs targeting human XDH "xanthine dehydrogenase" gene (human NCBI refseqID: NM_000379; NCBI GeneID: 7498) were designed using custom R and Python scripts. The human XDH REFSEQ mRNA has a length of 5717 bases. The rationale and method for the set of siRNA designs is as follows: the predicted efficacy for every potential 19mer siRNA from position 10 through position 5717 was determined with a linear model derived the direct measure of mRNA knockdown from more than 20,000 distinct siRNA designs targeting a large number of vertebrate genes. The custom Python script built the set of siRNAs by systematically selecting a siRNA every 11 bases along the target mRNA starting at position 10. At each of the positions, the neighboring siRNA (one position to the 5' end of the mRNA, one position to the 3' end of the mRNA) was swapped into the design set if the predicted efficacy was better than the efficacy at the exact every-11th siRNA. Low complexity siRNAs, i.e., those with Shannon Entropy measures below 1.35 were excluded from the set.

A detailed list of the unmodified XDH sense and antisense strand sequences is shown in Table 6 and a detailed list of the modified XDH sense and antisense strand sequences is shown in Table 7.

Cell Culture and Transfections

Primary human hepatocytes were thawed and cultured in WMEM with 5% FBS and maintenance reagents (Invitrogen, Carlsbad Calif. Cat #CM3000) on collagen-coated plates. After 24 hours, media was removed and replaced with 40 μl of WMEM with maintenance reagents (Inivtrogen, Carlsbad Calif. Cat #CM4000) containing ~5×10^3 cells. Separately, 7.35 μl of Opti-MEM plus 0.15 μl of Lipofectamine RNAiMax per well (Invitrogen, Carlsbad Calif. cat #13778-150) was added to 7.5 μl of siRNA duplexe and incubated at room temperature for 20 minutes. Ten μl of lipoplex mixture was then transferred to the cells. Cells were incubated for 24 hours prior to RNA purification. Single dose experiments were performed at 10 nM final duplex concentration.

Total RNA Isolation Using DYNABEADS mRNA Isolation Kit:

RNA was isolated using an automated protocol on a BioTek-EL406 platform using DYNABEADs (Invitrogen, cat #61012). Briefly, 50 μl of Lysis/Binding Buffer and 25 μl of lysis buffer containing 3 μl of magnetic beads were added to the plate with cells. Plates were incubated on an electromagnetic shaker for 10 minutes at room temperature and then magnetic beads were captured and the supernatant was removed. Bead-bound RNA was then washed 2 times with 150 μl Wash Buffer A and once with Wash Buffer B. Beads were then washed with 150 μl Elution Buffer, re-captured and supernatant removed.

cDNA Synthesis Using ABI High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, Calif., Cat #4368813):

Ten μl of a master mix containing 1 μl 10× Buffer, 0.4 μl 25× dNTPs, 1 μl 10× Random primers, 0.5 μl Reverse Transcriptase, 0.5 μl RNase inhibitor and 6.6 μl of H_2O per reaction was added to RNA isolated above. Plates were sealed, mixed, and incubated on an electromagnetic shaker for 10 minutes at room temperature, followed by 2 hours at 37° C.

Real Time PCR:

Two μl of cDNA were added to a master mix containing 0.5 μl of GAPDH TaqMan Probe (4326317E), 0.5 μl XDH probe (Hs00166010_m1) and 50 Lightcycler 480 probe master mix (Roche Cat #04887301001) per well in a 384 well plates (Roche cat #04887301001). Real time PCR was done in a LightCycler480 Real Time PCR system (Roche) using the ΔΔCt(RQ) assay. Each duplex was tested in four independent transfections.

To calculate relative fold change, real time data was analyzed using the ΔΔCt method and normalized to assays performed with mock transfected cells.

Table 8 shows the results of a single dose screen in Primary Human Hepatocytes transfected with the indicated iRNAs.

TABLE 6

XDH Unmodified Sequences

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO	Position in NM_000379.3	Antisense Oligo Name	Antisense Sequence (5'-3')	SEQ ID NO	Position in NM_000379.3
AD-71930	A-143855	ACUACCUGCCAGUGUCUCU	363	18-36	A-143856	AGAGACACUGGCAGGUAGU	677	18-36
AD-71931	A-143857	UUAGGAGUGAGGUACCUGA	364	36-54	A-143858	UCAGGUACCUCACUCCUAA	678	36-54
AD-71932	A-143861	AACCUGUGACAAUGACAGA	365	69-87	A-143862	UCUGUCAUUGUCACAGGUU	679	69-87
AD-71933	A-143863	GCAGACAAAUUGGUUUUCU	366	86-104	A-143864	AGAAAACCAAUUUGUCUGC	680	86-104
AD-71934	A-143865	UUUGUGAAUGGCAGAAAGA	367	104-122	A-143866	UCUUUCUGCCAUUCACAAA	681	104-122
AD-71935	A-143867	AAGGUGGUGGAGAAAAAUA	368	119-137	A-143868	UAUUUUUCUCCACCACCUU	682	119-137
AD-71936	A-143871	CCUUUUGGCCUACCUGAGA	369	154-172	A-143872	UCUCAGGUAGGCCAAAAGG	683	154-172
AD-71937	A-143873	AAGAAAGUUGGGGCUGAGU	370	172-190	A-143874	ACUCAGCCCCAACUUUCUU	684	172-190
AD-71938	A-143875	AGUGGAACCAAGCUCGGCU	371	188-206	A-143876	AGCCGAGCUUGGUUCCACU	685	188-206
AD-71939	A-143877	CUGUGGAGAGGGGGGCUGA	372	205-223	A-143878	UCAGCCCCCCUCUCCACAG	686	205-223
AD-71940	A-143879	CGGGGCUUGCACAGUGAUA	373	223-241	A-143880	UAUCACUGUGCAAGCCCCG	687	223-241
AD-71941	A-143881	AUGCUCUCCAAGUAUGAUA	374	239-257	A-143882	UAUCAUACUUGGAGAGCAU	688	239-257
AD-71942	A-143885	UCGUCCACUUUUCUGCCAA	375	273-291	A-143886	UUGGCAGAAAAGUGGACGA	689	273-291
AD-71943	A-143887	AUGCCUGCCUGGCCCCCAU	376	291-309	A-143888	AUGGGGGCCAGGCAGGCAU	690	291-309
AD-71944	A-143889	AUCUGCUCCUUGCACCAUA	377	308-326	A-143890	UAUGGUGCAAGGAGCAGAU	691	308-326
AD-71945	A-143891	UGUUGCAGUGACAACUGUA	378	325-343	A-143892	UACAGUUGUCACUGCAACA	692	325-343
AD-71946	A-143893	UGGAAGGAAUAGGAAGCAA	379	342-360	A-143894	UUGCUUCCUAUUCCUUCCA	693	342-360
AD-71947	A-143895	CACCAAGACGAGGCUGCAU	380	358-376	A-143896	AUGCAGCCUCGUCUUGGUG	694	358-376
AD-71948	A-143897	UCCUGUGCAGGAGAGAAUU	381	376-394	A-143898	AAUUCUCUCCUGCACAGGA	695	376-394
AD-71949	A-143899	AUUGCCAAAAGCCACGGCU	382	392-410	A-143900	AGCCGUGGCUUUUGGCAAU	696	392-410
AD-71950	A-143901	UCCCAGUGCGGGUUCUGCA	383	410-428	A-143902	UGCAGAACCCGCACUGGGA	697	410-428
AD-71951	A-143903	UGCACCCCUGGCAUCGUCA	384	425-443	A-143904	UGACGAUGCCAGGGGUGCA	698	425-443
AD-71952	A-143905	UGAGUAUGUACACACUGCU	385	444-462	A-143906	AGCAGUGUGUACAUACUCA	699	444-462
AD-71953	A-143907	UGCUCCGGAAUCAGCCCGA	386	459-477	A-143908	UCGGGCUGAUUCCGGAGCA	700	459-477
AD-71954	A-143909	AGCCCACCAUGGAGGAGAU	387	477-495	A-143910	AUCUCCUCCAUGGUGGGCU	701	477-495
AD-71955	A-143911	UUGAGAAUGCCUUCCAAGA	388	495-513	A-143912	UCUUGGAAGGCAUUCUCAA	702	495-513
AD-71956	A-143913	AAGGAAAUCUGUGCCGCUA	389	510-528	A-143914	UAGCGGCACAGAUUUCCUU	703	510-528
AD-71957	A-143915	CACAGGCUACAGACCCAUA	390	529-547	A-143916	UAUGGGUCUGUAGCCUGUG	704	529-547
AD-71958	A-143917	CAUCCUCCAGGGCUUCCGA	391	544-562	A-143918	UCGGAAGCCCUGGAGGAUG	705	544-562
AD-71959	A-143919	ACCUUUGCCAGGGAUGGUA	392	563-581	A-143920	UACCAUCCCUGGCAAAGGU	706	563-581
AD-71960	A-143921	UGGAUGCUGUGGAGGAGAU	393	580-598	A-143922	AUCUCCUCCACAGCAUCCA	707	580-598
AD-71961	A-143923	GAUGGGAAUAAUCCAAAUU	394	596-614	A-143924	AAUUUGGAUUAUUCCCAUC	708	596-614
AD-71962	A-143927	AGAAAGACCACUCAGUCAA	395	630-648	A-143928	UUGACUGAGUGGUCUUUCU	709	630-648
AD-71963	A-143929	AGCCUCUCGCCAUCUUUAU	396	647-665	A-143930	AUAAAGAUGGCGAGAGGCU	710	647-665
AD-71964	A-143931	UAUUCAAACCAGAGGAGUU	397	663-681	A-143932	AACUCCUCUGGUUUGAAUA	711	663-681
AD-71965	A-143933	UUCACGCCCCUGGAUCCAA	398	680-698	A-143934	UUGGAUCCAGGGGCGUGAA	712	680-698
AD-71966	A-143935	AACCCAGGAGCCCAUUUUU	399	697-715	A-143936	AAAAAUGGGCUCCUGGGUU	713	697-715

TABLE 6-continued

XDH Unmodified Sequences

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO	Position in NM_000379.3	Antisense Oligo Name	Antisense Sequence (5'-3')	SEQ ID NO	Position in NM_000379.3
AD-71967	A-143937	UUCCCCCAGAGUUGCUGAA	400	714-732	A-143938	UUCAGCAACUCUGGGGGAA	714	714-732
AD-71968	A-143939	AGGCUGAAAGACACUCCUA	401	731-749	A-143940	UAGGAGUGUCUUUCAGCCU	715	731-749
AD-71969	A-143941	UCGGAAGCAGCUGCGAUUU	402	748-766	A-143942	AAAUCGCAGCUGCUUCCGA	716	748-766
AD-71970	A-143943	UUGAAGGGGAGCGUGUGAA	403	765-783	A-143944	UUCACACGCUCCCCUUCAA	717	765-783
AD-71971	A-143945	CGUGGAUACAGGCCUCAAA	404	783-801	A-143946	UUUGAGGCCUGUAUCCACG	718	783-801
AD-71972	A-143947	AACCCUCAAGGAGCUGCUA	405	799-817	A-143948	UAGCAGCUCCUUGAGGGUU	719	799-817
AD-71973	A-143949	UGGACCUCAAGGCUCAGCA	406	816-834	A-143950	UGCUGAGCCUUGAGGUCCA	720	816-834
AD-71974	A-143951	ACCCUGACGCCAAGCUGGU	407	834-852	A-143952	ACCAGCUUGGCGUCAGGGU	721	834-852
AD-71975	A-143953	UCGUGGGGAACACGGAGAU	408	852-870	A-143954	AUCUCCGUGUUCCCCACGA	722	852-870
AD-71976	A-143955	AUUGGCAUUGAGAUGAAGU	409	869-887	A-143956	ACUUCAUCUCAAUGCCAAU	723	869-887
AD-71977	A-143957	AAGUUCAAGAAUAUGCUGU	410	884-902	A-143958	ACAGCAUAUUCUUGAACUU	724	884-902
AD-71978	A-143959	UUUCCUAUGAUUGUCUGCA	411	902-920	A-143960	UGCAGACAAUCAUAGGAAA	725	902-920
AD-71979	A-143961	CCCAGCCUGGAUCCCUGAA	412	919-937	A-143962	UUCAGGGAUCCAGGCUGGG	726	919-937
AD-71980	A-143963	AGCUGAAUUCGGUAGAACA	413	936-954	A-143964	UGUUCUACCGAAUUCAGCU	727	936-954
AD-71981	A-143965	CAUGGACCCGACGGUAUCU	414	953-971	A-143966	AGAUACCGUCGGGUCCAUG	728	953-971
AD-71982	A-143967	UCUCCUUUGGAGCUGCUUA	415	969-987	A-143968	UAAGCAGCUCCAAAGGAGA	729	969-987
AD-71983	A-143969	UGCCCCCUGAGCAUUGUGA	416	986-1004	A-143970	UCACAAUGCUCAGGGGGCA	730	986-1004
AD-71984	A-143971	AAAAAACCCUGGUGGAUGA	417	1005-1023	A-143972	UCAUCCACCAGGGUUUUUU	731	1005-1023
AD-71985	A-143973	UGCUGUUGCUAAGCUUCCU	418	1021-1039	A-143974	AGGAAGCUUAGCAACAGCA	732	1021-1039
AD-71986	A-143975	CCUGCCCAAAAGACAGAGA	419	1037-1055	A-143976	UCUCUGUCUUUUGGGCAGG	733	1037-1055
AD-71987	A-143977	UGUUCAGAGGGGUCCUGGA	420	1056-1074	A-143978	UCCAGGACCCCUCUGAACA	734	1056-1074
AD-71988	A-143979	UGGAGCAGCUGCGCUGGUU	421	1071-1089	A-143980	AACCAGCGCAGCUGCUCCA	735	1071-1089
AD-71989	A-143981	UUUGCUGGGAAGCAAGUCA	422	1088-1106	A-143982	UGACUUGCUUCCCAGCAAA	736	1088-1106
AD-71990	A-143983	CAAGUCUGUGGCGUCCGUU	423	1105-1123	A-143984	AACGGACGCCACAGACUUG	737	1105-1123
AD-71991	A-143985	UGGAGGGAACAUCAUCACU	424	1123-1141	A-143986	AGUGAUGAUGUUCCCUCCA	738	1123-1141
AD-71992	A-143987	UGCCAGCCCCAUCUCCGAA	425	1141-1159	A-143988	UUCGGAGAUGGGGCUGGCA	739	1141-1159
AD-71993	A-143989	ACCUCAACCCCGUGUUCAU	426	1158-1176	A-143990	AUGAACACGGGGUUGAGGU	740	1158-1176
AD-71994	A-143991	UCAUGGCCAGUGGGGCCAA	427	1173-1191	A-143992	UUGGCCCCACUGGCCAUGA	741	1173-1191
AD-71995	A-143993	AAGCUGACACUUGUGUCCA	428	1190-1208	A-143994	UGGACACAAGUGUCAGCUU	742	1190-1208
AD-71996	A-143995	CAGAGGCACCAGGAGAACU	429	1207-1225	A-143996	AGUUCUCCUGGUGCCUCUG	743	1207-1225
AD-71997	A-143997	UGUCCAGAUGGACCACACA	430	1225-1243	A-143998	UGUGUGGUCCAUCUGGACA	744	1225-1243
AD-71998	A-143999	CCUUCUUCCCUGGCUACAA	431	1242-1260	A-144000	UUGUAGCCAGGGAAGAAGG	745	1242-1260
AD-71999	A-144001	AGAAAGACCCUGCUGAGCA	432	1259-1277	A-144002	UGCUCAGCAGGGUCUUUCU	746	1259-1277
AD-72000	A-144003	CCGGAGGAGAUACUGCUCU	433	1277-1295	A-144004	AGAGCAGUAUCUCCUCCGG	747	1277-1295
AD-72001	A-144005	CUCUCCAUAGAGAUCCCCU	434	1292-1310	A-144006	AGGGGAUCUCUAUGGAGAG	748	1292-1310
AD-72002	A-144007	UACAGCAGGGAGGGGGAGU	435	1310-1328	A-144008	ACUCCCCCUCCCUGCUGUA	749	1310-1328
AD-72003	A-144009	AGUAUUUCUCAGCAUUCAA	436	1326-1344	A-144010	UUGAAUGCUGAGAAAUACU	750	1326-1344

TABLE 6-continued

XDH Unmodified Sequences

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO	Position in NM_000379.3	Antisense Oligo Name	Antisense Sequence (5'-3')	SEQ ID NO	Position in NM_000379.3
AD-72004	A-144011	AAGCAGGCCUCCCGGAGAA	437	1343-1361	A-144012	UUCUCCGGGAGGCCUGCUU	751	1343-1361
AD-72005	A-144013	AAGAUGACAUUGCCAAGGU	438	1362-1380	A-144014	ACCUUGGCAAUGUCAUCUU	752	1362-1380
AD-72006	A-144015	GGUAACCAGUGGCAUGAGA	439	1378-1396	A-144016	UCUCAUGCCACUGGUUACC	753	1378-1396
AD-72007	A-144017	AGAGUUUUAUUCAAGCCAA	440	1394-1412	A-144018	UUGGCUUGAAUAAAACUCU	754	1394-1412
AD-72008	A-144019	AGGAACCACAGAGGUACAA	441	1411-1429	A-144020	UUGUACCUCUGUGGUUCCU	755	1411-1429
AD-72009	A-144021	AGGAGCUGGCCCUUUGCUA	442	1428-1446	A-144022	UAGCAAAGGGCCAGCUCCU	756	1428-1446
AD-72010	A-144023	UAUGGUGGAAUGGCCAACA	443	1445-1463	A-144024	UGUUGGCCAUUCCACCAUA	757	1445-1463
AD-72011	A-144025	AGAACCAUCUCAGCCCUCA	444	1463-1481	A-144026	UGAGGGCUGAGAUGGUUCU	758	1463-1481
AD-72012	A-144027	AAGACCACUCAGAGGCAGA	445	1481-1499	A-144028	UCUGCCUCUGAGUGGUCUU	759	1481-1499
AD-72013	A-144029	CAGCUUUCCAAGCUCUGGA	446	1496-1514	A-144030	UCCAGAGCUUGGAAAGCUG	760	1496-1514
AD-72014	A-144031	AGGAGGAGCUGCUGCAGGA	447	1515-1533	A-144032	UCCUGCAGCAGCUCCUCCU	761	1515-1533
AD-72015	A-144033	AGGACGUGUGUGCAGGACU	448	1530-1548	A-144034	AGUCCUGCACACACGUCCU	762	1530-1548
AD-72016	A-144035	UGGCAGAGGAGCUGCAUCU	449	1548-1566	A-144036	AGAUGCAGCUCCUCUGCCA	763	1548-1566
AD-72017	A-144037	UCUGCCUCCCGAUGCCCCU	450	1564-1582	A-144038	AGGGGCAUCGGGAGGCAGA	764	1564-1582
AD-72018	A-144039	GGUGGCAUGGUGGACUUCA	451	1583-1601	A-144040	UGAAGUCCACCAUGCCACC	765	1583-1601
AD-72019	A-144041	UUCCGGUGCACCCUCACCA	452	1598-1616	A-144042	UGGUGAGGGUGCACCGGAA	766	1598-1616
AD-71752	A-144043	UCAGCUUCUUCUUCAAGUU	453	1617-1635	A-144044	AACUUGAAGAAGAAGCUGA	767	1617-1635
AD-71753	A-144045	UUCUACCUGACAGUCCUUA	454	1634-1652	A-144046	UAAGGACUGUCAGGUAGAA	768	1634-1652
AD-71754	A-144049	GAGAACCUGGAAGACAAGU	455	1667-1685	A-144050	ACUUGUCUUCCAGGUUCUC	769	1667-1685
AD-71755	A-144051	UGUGGUAAACUGGACCCCA	456	1685-1703	A-144052	UGGGGUCCAGUUUACCACA	770	1685-1703
AD-71756	A-144053	CACUUUCGCCAGUGCAACU	457	1702-1720	A-144054	AGUUGCACUGGCGAAAGUG	771	1702-1720
AD-71757	A-144055	ACUUUACUGUUUCAGAAAG	458	1718-1736	A-144056	CUUUCUGAAACAGUAAAGU	772	1718-1736
AD-71758	A-144057	AAGACCCCCCAGCCGAUGU	459	1734-1752	A-144058	ACAUCGGCUGGGGGGUCUU	773	1734-1752
AD-71759	A-144059	UCCAGCUCUUCCAAGAGGU	460	1752-1770	A-144060	ACCUCUUGGAAGAGCUGGA	774	1752-1770
AD-71760	A-144061	UGCCCAAGGGUCAGUCUGA	461	1770-1788	A-144062	UCAGACUGACCCUUGGGCA	775	1770-1788
AD-71761	A-144063	GAGGAGGACAUGGUGGGCA	462	1787-1805	A-144064	UGCCCACCAUGUCCUCCUC	776	1787-1805
AD-71762	A-144065	GCCGGCCCCUGCCCCACCU	463	1803-1821	A-144066	AGGUGGGGCAGGGGCCGGC	777	1803-1821
AD-71763	A-144067	CCUGGCAGCGGACAUGCAA	464	1819-1837	A-144068	UUGCAUGUCCGCUGCCAGG	778	1819-1837
AD-71764	A-144069	AGGCCUCUGGUGAGGCCGU	465	1836-1854	A-144070	ACGGCCUCACCAGAGGCCU	779	1836-1854
AD-71765	A-144071	UGUACUGUGACGACAUUCA	466	1854-1872	A-144072	UGAAUGUCGUCACAGUACA	780	1854-1872
AD-71766	A-144073	CUCGCUACGAGAAUGAGCU	467	1872-1890	A-144074	AGCUCAUUCUCGUAGCGAG	781	1872-1890
AD-71767	A-144075	CUGUCUCUCCGGCUGGUCA	468	1889-1907	A-144076	UGACCAGCCGGAGAGACAG	782	1889-1907
AD-71768	A-144079	CCACGCCAAGAUCAAGUCA	469	1921-1939	A-144080	UGACUUGAUCUUGGCGUGG	783	1921-1939
AD-71769	A-144081	CAUAGAUACAUCAGAAGCU	470	1939-1957	A-144082	AGCUUCUGAUGUAUCUAUG	784	1939-1957
AD-71770	A-144085	UUUGUUUGUUUCAUUUCCA	471	1973-1991	A-144086	UGGAAAUGAAACAAACAAA	785	1973-1991
AD-71771	A-144087	GCUGAUGAUGUUCCUGGGA	472	1991-2009	A-144088	UCCCAGGAACAUCAUCAGC	786	1991-2009
AD-71772	A-144093	CAGUCUUUGCGAAGGAUAA	473	2040-2058	A-144094	UUAUCCUUCGCAAAGACUG	787	2040-2058

TABLE 6-continued

XDH Unmodified Sequences

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO	Position in NM_000379.3	Antisense Oligo Name	Antisense Sequence (5'-3')	SEQ ID NO	Position in NM_000379.3
AD-71773	A-144095	AAGGUUACUUGUGUUGGGA	474	2057-2075	A-144096	UCCCAACACAAGUAACCUU	788	2057-2075
AD-71774	A-144097	CAUAUCAUUGGUGCUGUGA	475	2075-2093	A-144098	UCACAGCACCAAUGAUAUG	789	2075-2093
AD-71775	A-144099	UGGUUGCUGACACCCCGGA	476	2091-2109	A-144100	UCCGGGGUGUCAGCAACCA	790	2091-2109
AD-71776	A-144101	ACACACACAGAGAGCUGCA	477	2110-2128	A-144102	UGCAGCUCUCUGUGUGUGU	791	2110-2128
AD-71777	A-144103	GCCCAAGGGGUGAAAAUCA	478	2126-2144	A-144104	UGAUUUUCACCCCUUGGGC	792	2126-2144
AD-71778	A-144105	UCACCUAUGAAGAACUACA	479	2142-2160	A-144106	UGUAGUUCUUCAUAGGUGA	793	2142-2160
AD-71779	A-144109	GAGGAUGCUAUAAAGAACA	480	2177-2195	A-144110	UGUUCUUUAUAGCAUCCUC	794	2177-2195
AD-71780	A-144111	CAACUCCUUUUAUGGACCU	481	2194-2212	A-144112	AGGUCCAUAAAAGGAGUUG	795	2194-2212
AD-71781	A-144113	CCUGAGCUGAAGAUCGAGA	482	2210-2228	A-144114	UCUCGAUCUUCAGCUCAGG	796	2210-2228
AD-71782	A-144115	AAAGGGGACCUAAAGAAGA	483	2228-2246	A-144116	UCUUCUUUAGGUCCCCUUU	797	2228-2246
AD-71783	A-144117	AGGGGUUUUCCGAAGCAGA	484	2244-2262	A-144118	UCUGCUUCGGAAAACCCCU	798	2244-2262
AD-71784	A-144119	UAAUGUUGUGUCAGGGGAA	485	2263-2281	A-144120	UUCCCCUGACACAACAUUA	799	2263-2281
AD-71785	A-144121	AGAUAUACAUCGGUGGCCA	486	2280-2298	A-144122	UGGCCACCGAUGUAUAUCU	800	2280-2298
AD-71786	A-144123	GCCAAGAGCACUUCUACCU	487	2295-2313	A-144124	AGGUAGAAGUGCUCUUGGC	801	2295-2313
AD-71787	A-144125	GGAGACUCACUGCACCAUU	488	2314-2332	A-144126	AAUGGUGCAGUGAGUCUCC	802	2314-2332
AD-71788	A-144127	UUGCUGUUCCAAAAGGCGA	489	2331-2349	A-144128	UCGCCUUUUGGAACAGCAA	803	2331-2349
AD-71789	A-144129	CGAGGCAGGGGAGAUGGAA	490	2347-2365	A-144130	UUCCAUCUCCCCUGCCUCG	804	2347-2365
AD-71790	A-144131	AGCUCUUUGUGUCUACACA	491	2364-2382	A-144132	UGUGUAGACACAAAGAGCU	805	2364-2382
AD-71791	A-144133	CAGAACACCAUGAAGACCA	492	2381-2399	A-144134	UGGUCUUCAUGGUGUUCUG	806	2381-2399
AD-71792	A-144135	CAGAGCUUUGUUGCAAAAA	493	2399-2417	A-144136	UUUUUGCAACAAAGCUCUG	807	2399-2417
AD-71793	A-144137	AAAAUGUUGGGGGUUCCAA	494	2414-2432	A-144138	UUGGAACCCCCAACAUUUU	808	2414-2432
AD-71794	A-144139	AGCAAACCGGAUUGUGGUU	495	2431-2449	A-144140	AACCACAAUCCGGUUUGCU	809	2431-2449
AD-71795	A-144141	UUCGAGUGAAGAGAAUGGA	496	2448-2466	A-144142	UCCAUUCUCUUCACUCGAA	810	2448-2466
AD-71796	A-144143	AGGAGGCUUUGGAGGCAAA	497	2467-2485	A-144144	UUUGCCUCCAAAGCCUCCU	811	2467-2485
AD-71797	A-144145	AAGGAGACCCGGAGCACUA	498	2483-2501	A-144146	UAGUGCUCCGGGUCUCCUU	812	2483-2501
AD-71798	A-144147	UGUGGUGUCCACGGCAGUA	499	2500-2518	A-144148	UACUGCCGUGGACACCACA	813	2500-2518
AD-71799	A-144149	UGGCCCUGGCUGCAUAUAA	500	2517-2535	A-144150	UUAUAUGCAGCCAGGGCCA	814	2517-2535
AD-71800	A-144153	UGCGAUGCAUGCUGGACCA	501	2550-2568	A-144154	UGGUCCAGCAUGCAUCGCA	815	2550-2568
AD-71801	A-144155	CGUGAUGAGGACAUGCUGA	502	2567-2585	A-144156	UCAGCAUGUCCUCAUCACG	816	2567-2585
AD-71802	A-144157	UAACUGGUGGCAGACAUCA	503	2586-2604	A-144158	UGAUGUCUGCCACCAGUUA	817	2586-2604
AD-71803	A-144159	UCCCUUCCUGGCCAGAUAA	504	2602-2620	A-144160	UUAUCUGGCCAGGAAGGGA	818	2602-2620
AD-71804	A-144161	UACAAGGUUGGCUUCAUGA	505	2618-2636	A-144162	UCAUGAAGCCAACCUUGUA	819	2618-2636
AD-71805	A-144163	AGACUGGGACAGUUGUGGA	506	2637-2655	A-144164	UCCACAACUGUCCCAGUCU	820	2637-2655
AD-71806	A-144165	GCUCUUGAGGUGGACCACU	507	2654-2672	A-144166	AGUGGUCCACCUCAAGAGC	821	2654-2672
AD-71807	A-144167	ACUUCAGCAAUGUGGGGAA	508	2670-2688	A-144168	UUCCCCACAUUGCUGAAGU	822	2670-2688
AD-71808	A-144169	GAACACCCAGGAUCUCUCU	509	2686-2704	A-144170	AGAGAGAUCCUGGGUGUUC	823	2686-2704
AD-71809	A-144171	CAGAGUAUUAUGGAACGAA	510	2705-2723	A-144172	UUCGUUCCAUAAUACUCUG	824	2705-2723

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XDH Unmodified Sequences

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO	Position in NM_000379.3	Antisense Oligo Name	Antisense Sequence (5'-3')	SEQ ID NO	Position in NM_000379.3
AD-71810	A-144173	AGCUUUAUUCCACAUGGAA	511	2722-2740	A-144174	UUCCAUGUGGAAUAAAGCU	825	2722-2740
AD-71811	A-144175	ACAACUGCUAUAAAAUCCA	512	2739-2757	A-144176	UGGAUUUUAUAGCAGUUGU	826	2739-2757
AD-71812	A-144177	UCCCCAACAUCCGGGGCAA	513	2754-2772	A-144178	UUGCCCCGGAUGUUGGGGA	827	2754-2772
AD-71813	A-144179	UGGGCGGCUGUGCAAAACA	514	2773-2791	A-144180	UGUUUUGCACAGCCGCCCA	828	2773-2791
AD-71814	A-144181	AACCAACCUUCCCUCCAAA	515	2788-2806	A-144182	UUUGGAGGGAAGGUUGGUU	829	2788-2806
AD-71815	A-144183	ACACGGCCUUCCGGGGCUU	516	2805-2823	A-144184	AAGCCCCGGAAGGCCGUGU	830	2805-2823
AD-71816	A-144185	UUGGGGGGCCCCAGGGGAU	517	2823-2841	A-144186	AUCCCCUGGGGCCCCCCAA	831	2823-2841
AD-71817	A-144187	AUGCUCAUUGCCGAGUGCU	518	2840-2858	A-144188	AGCACUCGGCAAUGAGCAU	832	2840-2858
AD-71818	A-144189	UGGAUGAGUGAAGUUGCAA	519	2858-2876	A-144190	UUGCAACUUCACUCAUCCA	833	2858-2876
AD-71819	A-144191	AGUGACCUGUGGGAUGCCU	520	2875-2893	A-144192	AGGCAUCCCACAGGUCACU	834	2875-2893
AD-71820	A-144193	CCUGCAGAGGAGGUGCGGA	521	2891-2909	A-144194	UCCGCACCUCCUCUGCAGG	835	2891-2909
AD-71821	A-144195	AGAAAAAACCUGUACAAAG	522	2909-2927	A-144196	CUUUGUACAGGUUUUUUCU	836	2909-2927
AD-71822	A-144197	AAAGAAGGGGACCUGACAA	523	2924-2942	A-144198	UUGUCAGGUCCCCUUCUUU	837	2924-2942
AD-71823	A-144199	ACACUUCAACCAGAAGCUU	524	2941-2959	A-144200	AAGCUUCUGGUUGAAGUGU	838	2941-2959
AD-71824	A-144201	UUGAGGGUUUCACCUUGCA	525	2958-2976	A-144202	UGCAAGGUGAAACCCUCAA	839	2958-2976
AD-71825	A-144203	CAGAUGCUGGGAAGAAUGA	526	2977-2995	A-144204	UCAUUCUUCCCAGCAUCUG	840	2977-2995
AD-71826	A-144205	UGCCUAGCAAGCUCUCAGU	527	2993-3011	A-144206	ACUGAGAGCUUGCUAGGCA	841	2993-3011
AD-71827	A-144207	UAUCAUGCUCGGAAGAGUA	528	3011-3029	A-144208	UACUCUUCCGAGCAUGAUA	842	3011-3029
AD-71828	A-144209	AGUGAGGUUGACAAGUUCA	529	3026-3044	A-144210	UGAACUUGUCAACCUCACU	843	3026-3044
AD-71829	A-144211	AACAAGGAGAAUUGUUGGA	530	3044-3062	A-144212	UCCAACAAUUCUCCUUGUU	844	3044-3062
AD-71830	A-144213	AAAAAGAGAGGAUUGUGCA	531	3062-3080	A-144214	UGCACAAUCCUCUCUUUUU	845	3062-3080
AD-71831	A-144215	CAUAAUUCCCACCAAGUUU	532	3079-3097	A-144216	AAACUUGGUGGGAAUUAUG	846	3079-3097
AD-71832	A-144217	UUUGGAAUAAGCUUUACAA	533	3095-3113	A-144218	UUGUAAAGCUUAUUCCAAA	847	3095-3113
AD-71833	A-144219	AGUUCCUUUUCUGAAUCAA	534	3112-3130	A-144220	UUGAUUCAGAAAAGGAACU	848	3112-3130
AD-71834	A-144221	AGGCAGGAGCCCUACUUCA	535	3129-3147	A-144222	UGAAGUAGGGCUCCUGCCU	849	3129-3147
AD-71835	A-144223	CAUGUGUACACAGAUGGCU	536	3146-3164	A-144224	AGCCAUCUGUGUACACAUG	850	3146-3164
AD-71836	A-144225	UCUGUGCUGCUGACCCACA	537	3164-3182	A-144226	UGUGGGUCAGCAGCACAGA	851	3164-3182
AD-71837	A-144229	GGCCAAGGCCUUCAUACCA	538	3197-3215	A-144230	UGGUAUGAAGGCCUUGGCC	852	3197-3215
AD-71838	A-144231	AAAAUGGUCCAGGUGGCCA	539	3215-3233	A-144232	UGGCCACCUGGACCAUUUU	853	3215-3233
AD-71839	A-144233	GCCAGUAGAGCUCUGAAAA	540	3230-3248	A-144234	UUUUCAGAGCUCUACUGGC	854	3230-3248
AD-71840	A-144235	AAUCCCCACCUCUAAGAUU	541	3247-3265	A-144236	AAUCUUAGAGGUGGGGAUU	855	3247-3265
AD-71841	A-144237	UAUAUCAGCGAGACAAGCA	542	3266-3284	A-144238	UGCUUGUCUCGCUGAUAUA	856	3266-3284
AD-71842	A-144239	AGCACUAACACUGUGCCCA	543	3281-3299	A-144240	UGGGCACAGUGUUAGUGCU	857	3281-3299
AD-71843	A-144241	CAACACCUCUCCCACGGCU	544	3298-3316	A-144242	AGCCGUGGGAGAGGUGUUG	858	3298-3316
AD-71844	A-144243	UGCCUCUGUCAGCGCUGAA	545	3316-3334	A-144244	UUCAGCGCUGACAGAGGCA	859	3316-3334
AD-71845	A-144245	ACCUCAAUGGACAGGCCGU	546	3333-3351	A-144246	ACGGCCUGUCCAUUGAGGU	860	3333-3351
AD-71846	A-144247	GUCUAUGCGGCUUGUCAGA	547	3350-3368	A-144248	UCUGACAAGCCGCAUAGAC	861	3350-3368

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XDH Unmodified Sequences

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO	Position in NM_000379.3	Antisense Oligo Name	Antisense Sequence (5'-3')	SEQ ID NO	Position in NM_000379.3
AD-71847	A-144249	AGACCAUCUUGAAAAGGCU	548	3366-3384	A-144250	AGCCUUUUCAAGAUGGUCU	862	3366-3384
AD-71848	A-144251	UGGAACCCUACAAGAAGAA	549	3384-3402	A-144252	UUCUUCUUGUAGGGUUCCA	863	3384-3402
AD-71849	A-144253	AAGAAUCCCAGUGGCUCCU	550	3401-3419	A-144254	AGGAGCCACUGGGAUUCUU	864	3401-3419
AD-71850	A-144255	UGGGAAGACUGGGUCACAA	551	3419-3437	A-144256	UUGUGACCCAGUCUUCCCA	865	3419-3437
AD-71851	A-144257	ACAGCUGCCUACAUGGACA	552	3434-3452	A-144258	UGUCCAUGUAGGCAGCUGU	866	3434-3452
AD-71852	A-144259	ACAGUGAGCUUGUCUGCCA	553	3452-3470	A-144260	UGGCAGACAAGCUCACUGU	867	3452-3470
AD-71853	A-144261	ACUGGGUUUUAUAGAACAA	554	3470-3488	A-144262	UUGUUCUAUAAAACCCAGU	868	3470-3488
AD-71854	A-144263	ACCCAAUCUGGGCUACAGA	555	3487-3505	A-144264	UCUGUAGCCCAGAUUGGGU	869	3487-3505
AD-71855	A-144265	CAGCUUUGAGACUAACUCA	556	3502-3520	A-144266	UGAGUUAGUCUCAAAGCUG	870	3502-3520
AD-71856	A-144267	GGGAACCCCUUCCACUACU	557	3521-3539	A-144268	AGUAGUGGAAGGGGUUCCC	871	3521-3539
AD-71857	A-144269	CUUCAGCUAUGGGGUGGCU	558	3538-3556	A-144270	AGCCACCCCAUAGCUGAAG	872	3538-3556
AD-71858	A-144271	CUUGCUCUGAAGUAGAAAU	559	3555-3573	A-144272	AUUUCUACUUCAGAGCAAG	873	3555-3573
AD-71859	A-144273	AAAUCGACUGCCUAACAGA	560	3570-3588	A-144274	UCUGUUAGGCAGUCGAUUU	874	3570-3588
AD-71860	A-144275	GAGAUCAUAAGAACCUCCA	561	3588-3606	A-144276	UGGAGGUUCUUAUGAUCUC	875	3588-3606
AD-71861	A-144277	GCACAGAUAUUGUCAUGGA	562	3606-3624	A-144278	UCCAUGACAAUAUCUGUGC	876	3606-3624
AD-71862	A-144279	UGGAUGUUGGCUCCAGUCU	563	3621-3639	A-144280	AGACUGGAGCCAACAUCCA	877	3621-3639
AD-71863	A-144281	UAAACCCUGCCAUUGAUAU	564	3639-3657	A-144282	AUAUCAAUGGCAGGGUUUA	878	3639-3657
AD-71864	A-144283	UUGGACAGGUGGAAGGGGA	565	3657-3675	A-144284	UCCCCUUCCACCUGUCCAA	879	3657-3675
AD-71865	A-144285	GGGCAUUUGUCCAGGGCCU	566	3672-3690	A-144286	AGGCCCUGGACAAAUGCCC	880	3672-3690
AD-71866	A-144287	UUGGCCUCUUCACCCUAGA	567	3690-3708	A-144288	UCUAGGGUGAAGAGGCCAA	881	3690-3708
AD-71867	A-144289	AGAGGAGCUACACUAUUCA	568	3706-3724	A-144290	UGAAUAGUGUAGCUCCUCU	882	3706-3724
AD-71868	A-144291	CCCCGAGGGGAGCCUGCAA	569	3724-3742	A-144292	UUGCAGGCUCCCCUCGGGG	883	3724-3742
AD-71869	A-144293	CACACCCGUGGCCCUAGCA	570	3740-3758	A-144294	UGCUAGGGCCACGGGUGUG	884	3740-3758
AD-71870	A-144295	ACCUACAAGAUCCCGGCAU	571	3758-3776	A-144296	AUGCCGGGAUCUUGUAGGU	885	3758-3776
AD-71871	A-144297	UUUGGCAGCAUCCCCAUUA	572	3776-3794	A-144298	UAAUGGGGAUGCUGCCAAA	886	3776-3794
AD-71872	A-144301	CCUGCUCCGCGACUGCCCA	573	3808-3826	A-144302	UGGGCAGUCGCGGAGCAGG	887	3808-3826
AD-71873	A-144303	CAACAAGAAGGCCAUCUAU	574	3826-3844	A-144304	AUAGAUGGCCUUCUUGUUG	888	3826-3844
AD-71874	A-144305	UAUGCAUCGAAGGCUGUUA	575	3842-3860	A-144306	UAACAGCCUUCGAUGCAUA	889	3842-3860
AD-71875	A-144307	UGGAGAGCCGCCCCUCUUA	576	3859-3877	A-144308	UAAGAGGGGCGGCUCUCCA	890	3859-3877
AD-71876	A-144309	UCCUGGCUGCUUCUAUCUU	577	3876-3894	A-144310	AAGAUAGAAGCAGCCAGGA	891	3876-3894
AD-71877	A-144311	UUCUUUGCCAUCAAAGAUA	578	3893-3911	A-144312	UAUCUUUGAUGGCAAAGAA	892	3893-3911
AD-71878	A-144313	CCAUCCGUGCAGCUCGAGA	579	3912-3930	A-144314	UCUCGAGCUGCACGGAUGG	893	3912-3930
AD-71879	A-144315	AGCUCAGCACACAGGUAAU	580	3928-3946	A-144316	AUUACCUGUGUGCUGAGCU	894	3928-3946
AD-71880	A-144317	AAUAACGUGAAGGAACUCU	581	3944-3962	A-144318	AGAGUUCCUUCACGUUAUU	895	3944-3962
AD-71881	A-144319	UUCCGGCUAGACAGCCCUA	582	3962-3980	A-144320	UAGGGCUGUCUAGCCGGAA	896	3962-3980
AD-71882	A-144321	UGCCACCCCGGAGAAGAUA	583	3979-3997	A-144322	UAUCUUCUCCGGGGUGGCA	897	3979-3997
AD-71883	A-144323	AUCCGCAAUGCCUGCGUGA	584	3995-4013	A-144324	UCACGCAGGCAUUGCGGAU	898	3995-4013

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XDH Unmodified Sequences

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO	Position in NM_000379.3	Antisense Oligo Name	Antisense Sequence (5'-3')	SEQ ID NO	Position in NM_000379.3
AD-71884	A-144325	GACAAGUUCACCACCCUGU	585	4013-4031	A-144326	ACAGGGUGGUGAACUUGUC	899	4013-4031
AD-71885	A-144327	UGUGUGUCACUGGUGUCCA	586	4029-4047	A-144328	UGGACACCAGUGACACACA	900	4029-4047
AD-71886	A-144329	AGAAAACUGCAAACCCUGA	587	4048-4066	A-144330	UCAGGGUUUGCAGUUUUCU	901	4048-4066
AD-71887	A-144331	CUGGUCUGUGAGGGUCUAA	588	4063-4081	A-144332	UUAGACCCUCACAGACCAG	902	4063-4081
AD-71888	A-144333	AAAGAGAGAGUCCUCAGCA	589	4080-4098	A-144334	UGCUGAGGACUCUCUCUUU	903	4080-4098
AD-71889	A-144335	AGAGUCUUCUUGUGCUGCA	590	4098-4116	A-144336	UGCAGCACAAGAAGACUCU	904	4098-4116
AD-71890	A-144337	GCCUUUGGGCUUCCAUGGA	591	4114-4132	A-144338	UCCAUGGAAGCCCAAAGGC	905	4114-4132
AD-71891	A-144341	CAGAACAUGGAUCUAUUAA	592	4150-4168	A-144342	UUAAUAGAUCCAUGUUCUG	906	4150-4168
AD-71892	A-144343	UUAAAGUCACAGAAUGACA	593	4165-4183	A-144344	UGUCAUUCUGUGACUUUAA	907	4165-4183
AD-71893	A-144345	AGACCUGUGAUUUGUCAAA	594	4183-4201	A-144346	UUUGACAAAUCACAGGUCU	908	4183-4201
AD-71894	A-144347	AGAUGGGAUUUGGAAGACA	595	4200-4218	A-144348	UGUCUUCCAAAUCCCAUCU	909	4200-4218
AD-71895	A-144349	AAGUGAAUGCAAUGGAAGA	596	4218-4236	A-144350	UCUUCCAUUGCAUUCACUU	910	4218-4236
AD-71896	A-144351	AAGAUUUUGAUCAAAAAUA	597	4233-4251	A-144352	UAUUUUUGAUCAAAAUCUU	911	4233-4251
AD-71897	A-144353	UGUAAUUUGUAAACACAAU	598	4250-4268	A-144354	AUUGUGUUUACAAAUUACA	912	4250-4268
AD-71898	A-144355	GAUAAGCAAAUUCAAAACU	599	4269-4287	A-144356	AGUUUUGAAUUUGCUUAUC	913	4269-4287
AD-71899	A-144357	ACUGUUAUGCCUAAAUGGU	600	4285-4303	A-144358	ACCAUUUAGGCAUAACAGU	914	4285-4303
AD-71900	A-144359	UGAAUAUGCAAUUAGGAUA	601	4303-4321	A-144360	UAUCCUAAUUGCAUAUUCA	915	4303-4321
AD-71901	A-144361	AUCAUUUUCUGUCUGUUUU	602	4319-4337	A-144362	AAAACAGACAGAAAAUGAU	916	4319-4337
AD-71902	A-144363	UUAAUCAUGUAUCUGGAAU	603	4336-4354	A-144364	AUUCCAGAUACAUGAUUAA	917	4336-4354
AD-71903	A-144365	AAUAGGGUCGGGAAGGGUU	604	4352-4370	A-144366	AACCCUUCCCGACCCUAUU	918	4352-4370
AD-71904	A-144367	UUUGUGCUAUUCCCCACUU	605	4369-4387	A-144368	AAGUGGGGAAUAGCACAAA	919	4369-4387
AD-71905	A-144369	UUACUGGACAGCCUGUAUA	606	4386-4404	A-144370	UAUACAGGCUGUCCAGUAA	920	4386-4404
AD-71906	A-144371	AACCUCAAGUUCUGAUGGU	607	4404-4422	A-144372	ACCAUCAGAACUUGAGGUU	921	4404-4422
AD-71907	A-144373	UGUCUGUCCUUUGAAGAGA	608	4422-4440	A-144374	UCUCUUCAAAGGACAGACA	922	4422-4440
AD-71908	A-144375	AGGAUUCCCACAAACCUCU	609	4438-4456	A-144376	AGAGGUUUGUGGGAAUCCU	923	4438-4456
AD-71909	A-144377	UAGAAGCUUAAACCGAAGU	610	4456-4474	A-144378	ACUUCGGUUUAAGCUUCUA	924	4456-4474
AD-71910	A-144379	AAGUUACUUUAAAUCGUGU	611	4471-4489	A-144380	ACACGAUUUAAAGUAACUU	925	4471-4489
AD-71911	A-144381	UGCCUUCCUGUGAAAGCCU	612	4489-4507	A-144382	AGGCUUUCACAGGAAGGCA	926	4489-4507
AD-71912	A-144383	CUGGCCUUCAAACCAAUGA	613	4506-4524	A-144384	UCAUUGGUUUGAAGGCCAG	927	4506-4524
AD-71913	A-144385	AACAGCAAAGCAUAACCUU	614	4524-4542	A-144386	AAGGUUAUGCUUUGCUGUU	928	4524-4542
AD-71914	A-144387	UUGAAUCUAUACUCAAAUU	615	4541-4559	A-144388	AAUUUGAGUAUAGAUUCAA	929	4541-4559
AD-71915	A-144389	UUUUGCAAUGAGGCAGUGA	616	4558-4576	A-144390	UCACUGCCUCAUUGCAAAA	930	4558-4576
AD-71916	A-144391	UGGGGUAAGGUUAAAUCCU	617	4574-4592	A-144392	AGGAUUUAACCUUACCCCA	931	4574-4592
AD-71917	A-144393	UCUAACCAUCUUUGAAUCA	618	4592-4610	A-144394	UGAUUCAAAGAUGGUUAGA	932	4592-4610
AD-71918	A-144395	AUCAUUGGAAAGAAUAAAG	619	4607-4625	A-144396	CUUUAUUCUUUCCAAUGAU	933	4607-4625
AD-71919	A-144397	AAUGAAACAAAUUCAAGGU	620	4626-4644	A-144398	ACCUUGAAUUUGUUUCAUU	934	4626-4644
AD-71920	A-144399	AGGUUAAUUGGAUCUGAUU	621	4641-4659	A-144400	AAUCAGAUCCAAUUAACCU	935	4641-4659

TABLE 6-continued

XDH Unmodified Sequences

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO	Position in NM_000379.3	Antisense Oligo Name	Antisense Sequence (5'-3')	SEQ ID NO	Position in NM_000379.3
AD-71921	A-144401	UUUGUGAAGCUGCAUAAAG	622	4659-4677	A-144402	CUUUAUGCAGCUUCACAAA	936	4659-4677
AD-71922	A-144403	AGCAAGAUUACUCUAUAAU	623	4676-4694	A-144404	AUUAUAGAGUAAUCUUGCU	937	4676-4694
AD-71923	A-144405	UACAAAAAUCCAACCAACU	624	4694-4712	A-144406	AGUUGGUUGGAUUUUUGUA	938	4694-4712
AD-71924	A-144407	ACUCAAUUAUUGAGCACGU	625	4710-4728	A-144408	ACGUGCUCAAUAAUUGAGU	939	4710-4728
AD-71925	A-144409	UACAAUGUUCUAGAUUUCU	626	4728-4746	A-144410	AGAAAUCUAGAACAUUGUA	940	4728-4746
AD-71926	A-144411	UUCUUUCCCUUCCUCUUUA	627	4743-4761	A-144412	UAAAGAGGAAGGGAAAGAA	941	4743-4761
AD-71927	A-144413	GAAGAGAAUAUUUGUAUUA	628	4761-4779	A-144414	UAAUACAAAUAUUCUCUUC	942	4761-4779
AD-71928	A-144415	UUCCAAAUACUCUUUGAGU	629	4777-4795	A-144416	ACUCAAAGAGUAUUUGGAA	943	4777-4795
AD-71929	A-144417	UAUUUACAAAAAAGAUUAU	630	4795-4813	A-144418	AUAAUCUUUUUUGUAAAUA	944	4795-4813
AD-72020	A-144419	UAUGUUUAAUCUUUACAUU	631	4811-4829	A-144420	AAUGUAAAGAUUAAACAUA	945	4811-4829
AD-72021	A-144421	UUUGAAGCCAAAGUAAUUU	632	4828-4846	A-144422	AAAUUACUUUGGCUUCAAA	946	4828-4846
AD-72022	A-144423	UUCCACCUAGAAAUGAUGA	633	4845-4863	A-144424	UCAUCAUUUCUAGGUGGAA	947	4845-4863
AD-72023	A-144425	UAUCAGUCCUGGCAUGGUA	634	4864-4882	A-144426	UACCAUGCCAGGACUGAUA	948	4864-4882
AD-72024	A-144427	UGGCUCACCCCUAUAAUCA	635	4881-4899	A-144428	UGAUUAUAGGGGUGAGCCA	949	4881-4899
AD-72025	A-144429	AUCCCAGCACUUUGGGAGA	636	4896-4914	A-144430	UCUCCCAAAGUGCUGGGAU	950	4896-4914
AD-72026	A-144431	CUAAGGCAGGAGAAUUGCU	637	4915-4933	A-144432	AGCAAUUCUCCUGCCUUAG	951	4915-4933
AD-72027	A-144433	UGCUUGAGCCCAGCAGUUU	638	4930-4948	A-144434	AAACUGCUGGGCUCAAGCA	952	4930-4948
AD-72028	A-144435	UUGAGACCAGCCUGGGCAA	639	4947-4965	A-144436	UUGCCCAGGCUGGUCUCAA	953	4947-4965
AD-72029	A-144437	ACAUAGAGAGCUCCUGUCU	640	4965-4983	A-144438	AGACAGGAGCUCUCUAUGU	954	4965-4983
AD-72030	A-144439	UCUUUAAAAAAAAUUUUUU	641	4981-4999	A-144440	AAAAAAUUUUUUUUAAAGA	955	4981-4999
AD-72031	A-144441	UUAAUUAGUUGGUCUUGAU	642	4999-5017	A-144442	AUCAAGACCAACUAAUUAA	956	4999-5017
AD-72032	A-144443	UAGUGCAUGCCUGUAGUCA	643	5017-5035	A-144444	UGACUACAGGCAUGCACUA	957	5017-5035
AD-72033	A-144445	CCCAACUACUUGAAAGGCU	644	5034-5052	A-144446	AGCCUUUCAAGUAGUUGGG	958	5034-5052
AD-72034	A-144447	CUGAGGUGGAGAGAUCAUU	645	5051-5069	A-144448	AAUGAUCUCUCCACCUCAG	959	5051-5069
AD-72035	A-144449	UUUGAGCUCAGGAGGUUGA	646	5068-5086	A-144450	UCAACCUCCUGAGCUCAAA	960	5068-5086
AD-72036	A-144451	UUGAGGCUGCAGUGAGCUA	647	5083-5101	A-144452	UAGCUCACUGCAGCCUCAA	961	5083-5101
AD-72037	A-144455	CUCCUGCCUGAGCGACUGA	648	5118-5136	A-144456	UCAGUCGCUCAGGCAGGAG	962	5118-5136
AD-72038	A-144457	UGAGCAAGAUCUUGUCUCU	649	5134-5152	A-144458	AGAGACAAGAUCUUGCUCA	963	5134-5152
AD-72039	A-144459	UGAAGAAAAAAAAAAGAAAU	650	5152-5170	A-144460	AUUUCUUUUUUUUUCUUCA	964	5152-5170
AD-72040	A-144461	UAAAAAUGCUGCUAUCAAA	651	5170-5188	A-144462	UUUGAUAGCAGCAUUUUUA	965	5170-5188
AD-72041	A-144463	AAAAUCAAGCCCAACCAGA	652	5186-5204	A-144464	UCUGGUUGGGCUUGAUUUU	966	5186-5204
AD-72042	A-144465	AGAGGUAGAAGAGCCAAGA	653	5202-5220	A-144466	UCUUGGCUCUUCUACCUCU	967	5202-5220
AD-72043	A-144467	AAGCCUGGGUUCUCAUCCU	654	5220-5238	A-144468	AGGAUGAGAACCCAGGCUU	968	5220-5238
AD-72044	A-144469	CUAGCUCUGUCUCUUCUGU	655	5237-5255	A-144470	ACAGAAGAGACAGAGCUAG	969	5237-5255
AD-72045	A-144473	UUGGACUGUCAAUUCCCCU	656	5272-5290	A-144474	AGGGGAAUUGACAGUCCAA	970	5272-5290
AD-72046	A-144475	CCUUCCUGUGAUCCAUUUU	657	5288-5306	A-144476	AAAAUGGAUCACAGGAAGG	971	5288-5306
AD-72047	A-144481	UCUCACGUCUUCUGCUUUA	658	5339-5357	A-144482	UAAAGCAGAAGACGUGAGA	972	5339-5357

TABLE 6-continued

XDH Unmodified Sequences

Duplex Name	Sense Oligo Name	Sense Sequence (5' to 3')	SEQ ID NO	Position in NM_000379.3	Antisense Oligo Name	Antisense Sequence (5'-3')	SEQ ID NO	Position in NM_000379.3
AD-72048	A-144485	UAUGACCUGAAAACUCCAA	659	5372-5390	A-144486	UUGGAGUUUUCAGGUCAUA	973	5372-5390
AD-72049	A-144487	UUACAUAAAGGAUCUGCAA	660	5391-5409	A-144488	UUGCAGAUCCUUUAUGUAA	974	5391-5409
AD-72050	A-144489	CAGCUAUCUAAGGCUUGGU	661	5407-5425	A-144490	ACCAAGCCUUAGAUAGCUG	975	5407-5425
AD-72051	A-144493	AUGAUACCUGGGUCUAAUA	662	5440-5458	A-144494	UAUUAGACCCAGGUAUCAU	976	5440-5458
AD-72052	A-144495	AACUCUGCUGAGAUCACCU	663	5459-5477	A-144496	AGGUGAUCUCAGCAGAGUU	977	5459-5477
AD-72053	A-144497	ACCUCAAGUUUCUGCGGUU	664	5474-5492	A-144498	AACCGCAGAAACUUGAGGU	978	5474-5492
AD-72054	A-144499	UUGGUAAAGAGAACAAGGA	665	5491-5509	A-144500	UCCUUGUUCUCUUUACCAA	979	5491-5509
AD-72055	A-144501	AAGAACAAACAUCCCUUUU	666	5510-5528	A-144502	AAAAGGGAUGUUUGUUCUU	980	5510-5528
AD-72056	A-144503	UUUUAUUGCUCCAAAUGGU	667	5525-5543	A-144504	ACCAUUUGGAGCAAUAAAA	981	5525-5543
AD-72057	A-144505	GAUUUAAUCCCUACAUGGU	668	5544-5562	A-144506	ACCAUGUAGGGAUUAAAUC	982	5544-5562
AD-72058	A-144507	GGUGCUGGGUGGACAAUGU	669	5560-5578	A-144508	ACAUUGUCCACCCAGCACC	983	5560-5578
AD-72059	A-144509	UGUCACUGUCACAUGCCUU	670	5578-5596	A-144510	AAGGCAUGUGACAGUGACA	984	5578-5596
AD-72060	A-144511	UUCACUGUAUAAAUCCAAA	671	5595-5613	A-144512	UUUGGAUUUAUACAGUGAA	985	5595-5613
AD-72061	A-144513	ACCUUCUGCCAGAGAGAAU	672	5612-5630	A-144514	AUUCUCUCUGGCAGAAGGU	986	5612-5630
AD-72062	A-144517	CAUGGAGGGAGGAUAGUGA	673	5644-5662	A-144518	UCACUAUCCUCCCUCCAUG	987	5644-5662
AD-72063	A-144519	AAAUGAUAUAGUUGGACUA	674	5663-5681	A-144520	UAGUCCAACUAUAUCAUUU	988	5663-5681
AD-72064	A-144521	ACUGGUGCUUGAUGUCACU	675	5678-5696	A-144522	AGUGACAUCAAGCACCAGU	989	5678-5696
AD-72065	A-144523	CUAAUAAAUGAAACUGUCA	676	5695-5713	A-144524	UGACAGUUUCAUUUAUUAG	990	5695-5713

TABLE 7

XDH Modified Sequences

Duplex Name	Sense Oligo Name	Sense OligoSeq	SEQ ID NO	Anti-sense Oligo Name	Antisense OligoSeq	SEQ ID NO	mRNA target sequence	SEQ ID NO
AD-71930	A-143855	ACUACCUGCCAG UGUCUCUdTdT	991	A-143856	AGAGACACUGGCAGGUAGUdTdT	1305	ACUACCUGCCAGUGUCUCU	1619
AD-71931	A-143857	UUAGGAGUGAGG UACCUGAdTdT	992	A-143858	UCAGGUACCUCACUCCUAAdTdT	1306	UUAGGAGUGAGGUACCUGG	1620
AD-71932	A-143861	AACCUGUGACAA UGACAGAdTdT	993	A-143862	UCUGUCAUUGUCACAGGUUdTdT	1307	AACCUGUGACAAUGACAGC	1621
AD-71933	A-143863	GCAGACAAAUUG GUUUUCUdTdT	994	A-143864	AGAAAACCAAUUUGUCUGCdTdT	1308	GCAGACAAAUUGGUUUUCU	1622
AD-71934	A-143865	UUUGUGAAUGGC AGAAAGAdTdT	995	A-143866	UCUUUCUGCCAUUCACAAAdTdT	1309	UUUGUGAAUGGCAGAAAGG	1623
AD-71935	A-143867	AAGGUGGUGGAG AAAAAUAdTdT	996	A-143868	UAUUUUUCUCCACCACCUUdTdT	1310	AAGGUGGUGGAGAAAAAUG	1624
AD-71936	A-143871	CCUUUUGGCCUA CCUGAGAdTdT	997	A-143872	UCUCAGGUAGGCCAAAAGGdTdT	1311	CCUUUUGGCCUACCUGAGA	1625
AD-71937	A-143873	AAGAAAGUUGGG GCUGAGUdTdT	998	A-143874	ACUCAGCCCCAACUUUCUUdTdT	1312	AAGAAAGUUGGGGCUGAGU	1626
AD-71938	A-143875	AGUGGAACCAAG CUCGGCUdTdT	999	A-143876	AGCCGAGCUUGGUUCCACUdTdT	1313	AGUGGAACCAAGCUCGGCU	1627

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XDH Modified Sequences

Duplex Name	Sense Oligo Name	Sense OligoSeq	SEQ ID NO	Anti-sense Oligo Name	Antisense OligoSeq	SEQ ID NO	mRNA target sequence	SEQ ID NO
AD-71939	A-143877	CUGUGGAGAGGGGGGCUGAdTdT	1000	A-143878	UCAGCCCCCCUCUCCACAGdTdT	1314	CUGUGGAGAGGGGGGCUGC	1628
AD-71940	A-143879	CGGGGCUUGCACAGUGAUAdTdT	1001	A-143880	UAUCACUGUGCAAGCCCCGdTdT	1315	CGGGGCUUGCACAGUGAUG	1629
AD-71941	A-143881	AUGCUCUCCAAGUAUGAUAdTdT	1002	A-143882	UAUCAUACUUGGAGAGCAUdTdT	1316	AUGCUCUCCAAGUAUGAUC	1630
AD-71942	A-143885	UCGUCCACUUUUCUGCCAAdTdT	1003	A-143886	UUGGCAGAAAAGUGGACGAdTdT	1317	UCGUCCACUUUUCUGCCAA	1631
AD-71943	A-143887	AUGCCUGCCUGGCCCCCAUdTdT	1004	A-143888	AUGGGGGCCAGGCAGGCAUdTdT	1318	AUGCCUGCCUGGCCCCCAU	1632
AD-71944	A-143889	AUCUGCUCCUUGCACCAUAdTdT	1005	A-143890	UAUGGUGCAAGGAGCAGAUdTdT	1319	AUCUGCUCCUUGCACCAUG	1633
AD-71945	A-143891	UGUUGCAGUGACAACUGUAdTdT	1006	A-143892	UACAGUUGUCACUGCAACAdTdT	1320	UGUUGCAGUGACAACUGUG	1634
AD-71946	A-143893	UGGAAGGAAUAGGAAGCAAdTdT	1007	A-143894	UUGCUUCCUAUUCCUUCCAdTdT	1321	UGGAAGGAAUAGGAAGCAC	1635
AD-71947	A-143895	CACCAAGACGAGGCUGCAUdTdT	1008	A-143896	AUGCAGCCUCGUCUUGGUGdTdT	1322	CACCAAGACGAGGCUGCAU	1636
AD-71948	A-143897	UCCUGUGCAGGAGAGAAUUdTdT	1009	A-143898	AAUUCUCUCCUGCACAGGAdTdT	1323	UCCUGUGCAGGAGAGAAUU	1637
AD-71949	A-143899	AUUGCCAAAAGCCACGGCUdTdT	1010	A-143900	AGCCGUGGCUUUUGGCAAUdTdT	1324	AUUGCCAAAAGCCACGGCU	1638
AD-71950	A-143901	UCCCAGUGCGGGUUCUGCAdTdT	1011	A-143902	UGCAGAACCCGCACUGGGAdTdT	1325	UCCCAGUGCGGGUUCUGCA	1639
AD-71951	A-143903	UGCACCCCUGGCAUCGUCAdTdT	1012	A-143904	UGACGAUGCCAGGGGUGCAdTdT	1326	UGCACCCCUGGCAUCGUCA	1640
AD-71952	A-143905	UGAGUAUGUACACACUGCUdTdT	1013	A-143906	AGCAGUGUGUACAUACUCAdTdT	1327	UGAGUAUGUACACACUGCU	1641
AD-71953	A-143907	UGCUCCGGAAUCAGCCCGAdTdT	1014	A-143908	UCGGGCUGAUUCCGGAGCAdTdT	1328	UGCUCCGGAAUCAGCCCGA	1642
AD-71954	A-143909	AGCCCACCAUGGAGGAGAUdTdT	1015	A-143910	AUCUCCUCCAUGGUGGGCUdTdT	1329	AGCCCACCAUGGAGGAGAU	1643
AD-71955	A-143911	UUGAGAAUGCCUUCCAAGAdTdT	1016	A-143912	UCUUGGAAGGCAUUCUCAAdTdT	1330	UUGAGAAUGCCUUCCAAGG	1644
AD-71956	A-143913	AAGGAAAUCUGUGCCGCUAdTdT	1017	A-143914	UAGCGGCACAGAUUUCCUUdTdT	1331	AAGGAAAUCUGUGCCGCUG	1645
AD-71957	A-143915	CACAGGCUACAGACCCAUAdTdT	1018	A-143916	UAUGGGUCUGUAGCCUGUGdTdT	1332	CACAGGCUACAGACCCAUC	1646
AD-71958	A-143917	CAUCCUCCAGGGCUUCCGAdTdT	1019	A-143918	UCGGAAGCCCUGGAGGAUGdTdT	1333	CAUCCUCCAGGGCUUCCGG	1647
AD-71959	A-143919	ACCUUUGCCAGGGAUGGUAdTdT	1020	A-143920	UACCAUCCCUGGCAAAGGUdTdT	1334	ACCUUUGCCAGGGAUGGUG	1648
AD-71960	A-143921	UGGAUGCUGUGGAGGAGAUdTdT	1021	A-143922	AUCUCCUCCACAGCAUCCAdTdT	1335	UGGAUGCUGUGGAGGAGAU	1649
AD-71961	A-143923	GAUGGGAAUAAUCCAAAUUdTdT	1022	A-143924	AAUUUGGAUUAUUCCCAUCdTdT	1336	GAUGGGAAUAAUCCAAAUU	1650
AD-71962	A-143927	AGAAAGACCACUCAGUCAAdTdT	1023	A-143928	UUGACUGAGUGGUCUUUCUdTdT	1337	AGAAAGACCACUCAGUCAG	1651

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XDH Modified Sequences

Duplex Name	Sense Oligo Name	Sense OligoSeq	SEQ ID NO	Anti-sense Oligo Name	Antisense OligoSeq	SEQ ID NO	mRNA target sequence	SEQ ID NO
AD-71963	A-143929	AGCCUCUCGCCA UCUUUAUdTdT	1024	A-143930	AUAAAGAUGGCGAGAGGCUdTdT	1338	AGCCUCUCGCCAUCUUUAU	1652
AD-71964	A-143931	UAUUCAAACCAG AGGAGUUdTdT	1025	A-143932	AACUCCUCUGGUUUGAAUAdTdT	1339	UAUUCAAACCAGAGGAGUU	1653
AD-71965	A-143933	UUCACGCCCCUG GAUCCAAdTdT	1026	A-143934	UUGGAUCCAGGGGCGUGAAdTdT	1340	UUCACGCCCCUGGAUCCAA	1654
AD-71966	A-143935	AACCCAGGAGCC CAUUUUUdTdT	1027	A-143936	AAAAAUGGGCUCCUGGGUUdTdT	1341	AACCCAGGAGCCCAUUUUU	1655
AD-71967	A-143937	UUCCCCCAGAGU UGCUGAAdTdT	1028	A-143938	UUCAGCAACUCUGGGGGAAdTdT	1342	UUCCCCCAGAGUUGCUGAG	1656
AD-71968	A-143939	AGGCUGAAAGAC ACUCCUAdTdT	1029	A-143940	UAGGAGUGUCUUUCAGCCUdTdT	1343	AGGCUGAAAGACACUCCUC	1657
AD-71969	A-143941	UCGGAAGCAGCU GCGAUUUdTdT	1030	A-143942	AAAUCGCAGCUGCUUCCGAdTdT	1344	UCGGAAGCAGCUGCGAUUU	1658
AD-71970	A-143943	UUGAAGGGGAGC GUGUGAAdTdT	1031	A-143944	UUCACACGCUCCCCUUCAAdTdT	1345	UUGAAGGGGAGCGUGUGAC	1659
AD-71971	A-143945	CGUGGAUACAGG CCUCAAAdTdT	1032	A-143946	UUUGAGGCCUGUAUCCACGdTdT	1346	CGUGGAUACAGGCCUCAAC	1660
AD-71972	A-143947	AACCCUCAAGGA GCUGCUAdTdT	1033	A-143948	UAGCAGCUCCUUGAGGGUUdTdT	1347	AACCCUCAAGGAGCUGCUG	1661
AD-71973	A-143949	UGGACCUCAAGG CUCAGCAdTdT	1034	A-143950	UGCUGAGCCUUGAGGUCCAdTdT	1348	UGGACCUCAAGGCUCAGCA	1662
AD-71974	A-143951	ACCCUGACGCCA AGCUGGUdTdT	1035	A-143952	ACCAGCUUGGCGUCAGGGUdTdT	1349	ACCCUGACGCCAAGCUGGU	1663
AD-71975	A-143953	UCGUGGGGAACA CGGAGAUdTdT	1036	A-143954	AUCUCCGUGUUCCCCACGAdTdT	1350	UCGUGGGGAACACGGAGAU	1664
AD-71976	A-143955	AUUGGCAUUGAG AUGAAGUdTdT	1037	A-143956	ACUUCAUCUCAAUGCCAAUdTdT	1351	AUUGGCAUUGAGAUGAAGU	1665
AD-71977	A-143957	AAGUUCAAGAAU AUGCUGUdTdT	1038	A-143958	ACAGCAUAUUCUUGAACUUdTdT	1352	AAGUUCAAGAAUAUGCUGU	1666
AD-71978	A-143959	UUUCCUAUGAUU GUCUGCAdTdT	1039	A-143960	UGCAGACAAUCAUAGGAAAdTdT	1353	UUUCCUAUGAUUGUCUGCC	1667
AD-71979	A-143961	CCCAGCCUGGAU CCCUGAAdTdT	1040	A-143962	UUCAGGGAUCCAGGCUGGGdTdT	1354	CCCAGCCUGGAUCCCUGAG	1668
AD-71980	A-143963	AGCUGAAUUCGG UAGAACAdTdT	1041	A-143964	UGUUCUACCGAAUUCAGCUdTdT	1355	AGCUGAAUUCGGUAGAACA	1669
AD-71981	A-143965	CAUGGACCCGAC GGUAUCUdTdT	1042	A-143966	AGAUACCGUCGGGUCCAUGdTdT	1356	CAUGGACCCGACGGUAUCU	1670
AD-71982	A-143967	UCUCCUUUGGAG CUGCUUAdTdT	1043	A-143968	UAAGCAGCUCCAAAGGAGAdTdT	1357	UCUCCUUUGGAGCUGCUUG	1671
AD-71983	A-143969	UGCCCCCUGAGC AUUGUGAdTdT	1044	A-143970	UCACAAUGCUCAGGGGGCAdTdT	1358	UGCCCCCUGAGCAUUGUGG	1672
AD-71984	A-143971	AAAAAACCCUGG UGGAUGAdTdT	1045	A-143972	UCAUCCACCAGGGUUUUUUdTdT	1359	AAAAAACCCUGGUGGAUGC	1673
AD-71985	A-143973	UGCUGUUGCUAA GCUUCCUdTdT	1046	A-143974	AGGAAGCUUAGCAACAGCAdTdT	1360	UGCUGUUGCUAAGCUUCCU	1674
AD-71986	A-143975	CCUGCCCAAAAG ACAGAGAdTdT	1047	A-143976	UCUCUGUCUUUUGGGCAGGdTdT	1361	CCUGCCCAAAAGACAGAGG	1675

TABLE 7-continued

XDH Modified Sequences

Duplex Name	Sense Oligo Name	Sense OligoSeq	SEQ ID NO	Anti-sense Oligo Name	Antisense OligoSeq	SEQ ID NO	mRNA target sequence	SEQ ID NO
AD-71987	A-143977	UGUUCAGAGGGG UCCUGGAdTdT	1048	A-143978	UCCAGGACCCCUCUGAACAdTdT	1362	UGUUCAGAGGGGUCCUGGA	1676
AD-71988	A-143979	UGGAGCAGCUGC GCUGGUUdTdT	1049	A-143980	AACCAGCGCAGCUGCUCCAdTdT	1363	UGGAGCAGCUGCGCUGGUU	1677
AD-71989	A-143981	UUUGCUGGGAAG CAAGUCAdTdT	1050	A-143982	UGACUUGCUUCCCAGCAAAdTdT	1364	UUUGCUGGGAAGCAAGUCA	1678
AD-71990	A-143983	CAAGUCUGUGGC GUCCGUUdTdT	1051	A-143984	AACGGACGCCACAGACUUGdTdT	1365	CAAGUCUGUGGCGUCCGUU	1679
AD-71991	A-143985	UGGAGGGAACAU CAUCACUdTdT	1052	A-143986	AGUGAUGAUGUUCCCUCCAdTdT	1366	UGGAGGGAACAUCAUCACU	1680
AD-71992	A-143987	UGCCAGCCCCAU CUCCGAAdTdT	1053	A-143988	UUCGGAGAUGGGGCUGGCAdTdT	1367	UGCCAGCCCCAUCUCCGAC	1681
AD-71993	A-143989	ACCUCAACCCCG UGUUCAUdTdT	1054	A-143990	AUGAACACGGGGUUGAGGUdTdT	1368	ACCUCAACCCCGUGUUCAU	1682
AD-71994	A-143991	UCAUGGCCAGUG GGGCCAAdTdT	1055	A-143992	UUGGCCCCACUGGCCAUGAdTdT	1369	UCAUGGCCAGUGGGGCCAA	1683
AD-71995	A-143993	AAGCUGACACUU GUGUCCAdTdT	1056	A-143994	UGGACACAAGUGUCAGCUUdTdT	1370	AAGCUGACACUUGUGUCCA	1684
AD-71996	A-143995	CAGAGGCACCAG GAGAACUdTdT	1057	A-143996	AGUUCUCCUGGUGCCUCUGdTdT	1371	CAGAGGCACCAGGAGAACU	1685
AD-71997	A-143997	UGUCCAGAUGGA CCACACAdTdT	1058	A-143998	UGUGUGGUCCAUCUGGACAdTdT	1372	UGUCCAGAUGGACCACACC	1686
AD-71998	A-143999	CCUUCUUCCCUG GCUACAAdTdT	1059	A-144000	UUGUAGCCAGGGAAGAAGGdTdT	1373	CCUUCUUCCCUGGCUACAG	1687
AD-71999	A-144001	AGAAAGACCCUG CUGAGCAdTdT	1060	A-144002	UGCUCAGCAGGGUCUUUCUdTdT	1374	AGAAAGACCCUGCUGAGCC	1688
AD-72000	A-144003	CCGGAGGAGAUA CUGCUCUdTdT	1061	A-144004	AGAGCAGUAUCUCCUCCGGdTdT	1375	CCGGAGGAGAUACUGCUCU	1689
AD-72001	A-144005	CUCUCCAUAGAG AUCCCCUdTdT	1062	A-144006	AGGGGAUCUCUAUGGAGAGdTdT	1376	CUCUCCAUAGAGAUCCCCU	1690
AD-72002	A-144007	UACAGCAGGGAG GGGGAGUdTdT	1063	A-144008	ACUCCCCCUCCCUGCUGUAdTdT	1377	UACAGCAGGGAGGGGGAGU	1691
AD-72003	A-144009	AGUAUUUCUCAG CAUUCAAdTdT	1064	A-144010	UUGAAUGCUGAGAAAUACUdTdT	1378	AGUAUUUCUCAGCAUUCAA	1692
AD-72004	A-144011	AAGCAGGCCUCC CGGAGAAdTdT	1065	A-144012	UUCUCCGGGAGGCCUGCUUdTdT	1379	AAGCAGGCCUCCCGGAGAG	1693
AD-72005	A-144013	AAGAUGACAUUG CCAAGGUdTdT	1066	A-144014	ACCUUGGCAAUGUCAUCUUdTdT	1380	AAGAUGACAUUGCCAAGGU	1694
AD-72006	A-144015	GGUAACCAGUGG CAUGAGAdTdT	1067	A-144016	UCUCAUGCCACUGGUUACCdTdT	1381	GGUAACCAGUGGCAUGAGA	1695
AD-72007	A-144017	AGAGUUUUAUUC AAGCCAAdTdT	1068	A-144018	UUGGCUUGAAUAAAACUCUdTdT	1382	AGAGUUUUAUUCAAGCCAG	1696
AD-72008	A-144019	AGGAACCACAGA GGUACAAdTdT	1069	A-144020	UUGUACCUCUGUGGUUCCUdTdT	1383	AGGAACCACAGAGGUACAG	1697
AD-72009	A-144021	AGGAGCUGGCCC UUUGCUAdTdT	1070	A-144022	UAGCAAAGGGCCAGCUCCUdTdT	1384	AGGAGCUGGCCCUUUGCUA	1698
AD-72010	A-144023	UAUGGUGGAAUG GCCAACAdTdT	1071	A-144024	UGUUGGCCAUUCCACCAUAdTdT	1385	UAUGGUGGAAUGGCCAACA	1699

TABLE 7-continued

XDH Modified Sequences

Duplex Name	Sense Oligo Name	Sense OligoSeq	SEQ ID NO	Anti-sense Oligo Name	Antisense OligoSeq	SEQ ID NO	mRNA target sequence	SEQ ID NO
AD-72011	A-144025	AGAACCAUCUCA GCCCUCAdTdT	1072	A-144026	UGAGGGCUGAGAUGGUUCUdTdT	1386	AGAACCAUCUCAGCCCUCA	1700
AD-72012	A-144027	AAGACCACUCAG AGGCAGAdTdT	1073	A-144028	UCUGCCUCUGAGUGGUCUUdTdT	1387	AAGACCACUCAGAGGCAGC	1701
AD-72013	A-144029	CAGCUUUCCAAG CUCUGGAdTdT	1074	A-144030	UCCAGAGCUUGGAAAGCUGdTdT	1388	CAGCUUUCCAAGCUCUGGA	1702
AD-72014	A-144031	AGGAGGAGCUGC UGCAGGAdTdT	1075	A-144032	UCCUGCAGCAGCUCCUCCUdTdT	1389	AGGAGGAGCUGCUGCAGGA	1703
AD-72015	A-144033	AGGACGUGUGUG CAGGACUdTdT	1076	A-144034	AGUCCUGCACACACGUCCUdTdT	1390	AGGACGUGUGUGCAGGACU	1704
AD-72016	A-144035	UGGCAGAGGAGC UGCAUCUdTdT	1077	A-144036	AGAUGCAGCUCCUCUGCCAdTdT	1391	UGGCAGAGGAGCUGCAUCU	1705
AD-72017	A-144037	UCUGCCUCCCGA UGCCCCUdTdT	1078	A-144038	AGGGGCAUCGGGAGGCAGAdTdT	1392	UCUGCCUCCCGAUGCCCCU	1706
AD-72018	A-144039	GGUGGCAUGGUG GACUUCAdTdT	1079	A-144040	UGAAGUCCACCAUGCCACCdTdT	1393	GGUGGCAUGGUGGACUUCC	1707
AD-72019	A-144041	UUCCGGUGCACC CUCACCAdTdT	1080	A-144042	UGGUGAGGGUGCACCGGAAdTdT	1394	UUCCGGUGCACCCUCACCC	1708
AD-71752	A-144043	UCAGCUUCUUCU UCAAGUUdTdT	1081	A-144044	AACUUGAAGAAGAAGCUGAdTdT	1395	UCAGCUUCUUCUUCAAGUU	1709
AD-71753	A-144045	UUCUACCUGACA GUCCUUAdTdT	1082	A-144046	UAAGGACUGUCAGGUAGAAdTdT	1396	UUCUACCUGACAGUCCUUC	1710
AD-71754	A-144049	GAGAACCUGGAA GACAAGUdTdT	1083	A-144050	ACUUGUCUUCCAGGUUCUCdTdT	1397	GAGAACCUGGAAGACAAGU	1711
AD-71755	A-144051	UGUGGUAAACUG GACCCCAdTdT	1084	A-144052	UGGGGUCCAGUUUACCACAdTdT	1398	UGUGGUAAACUGGACCCCA	1712
AD-71756	A-144053	CACUUUCGCCAG UGCAACUdTdT	1085	A-144054	AGUUGCACUGGCGAAAGUGdTdT	1399	CACUUUCGCCAGUGCAACU	1713
AD-71757	A-144055	ACUUUACUGUUU CAGAAAGdTdT	1086	A-144056	CUUUCUGAAACAGUAAAGUdTdT	1400	ACUUUACUGUUUCAGAAAG	1714
AD-71758	A-144057	AAGACCCCCCAG CCGAUGUdTdT	1087	A-144058	ACAUCGGCUGGGGGGUCUUdTdT	1401	AAGACCCCCCAGCCGAUGU	1715
AD-71759	A-144059	UCCAGCUCUUCC AAGAGGUdTdT	1088	A-144060	ACCUCUUGGAAGAGCUGGAdTdT	1402	UCCAGCUCUUCCAAGAGGU	1716
AD-71760	A-144061	UGCCCAAGGGUC AGUCUGAdTdT	1089	A-144062	UCAGACUGACCCUUGGGCAdTdT	1403	UGCCCAAGGGUCAGUCUGA	1717
AD-71761	A-144063	GAGGAGGACAUG GUGGGCAdTdT	1090	A-144064	UGCCCACCAUGUCCUCCUCdTdT	1404	GAGGAGGACAUGGUGGGCC	1718
AD-71762	A-144065	GCCGGCCCCUGC CCCACCUdTdT	1091	A-144066	AGGUGGGGCAGGGGCCGGCdTdT	1405	GCCGGCCCCUGCCCCACCU	1719
AD-71763	A-144067	CCUGGCAGCGGA CAUGCAAdTdT	1092	A-144068	UUGCAUGUCCGCUGCCAGGdTdT	1406	CCUGGCAGCGGACAUGCAG	1720
AD-71764	A-144069	AGGCCUCUGGUG AGGCCGUdTdT	1093	A-144070	ACGGCCUCACCAGAGGCCUdTdT	1407	AGGCCUCUGGUGAGGCCGU	1721
AD-71765	A-144071	UGUACUGUGACG ACAUUCAdTdT	1094	A-144072	UGAAUGUCGUCACAGUACAdTdT	1408	UGUACUGUGACGACAUUCC	1722
AD-71766	A-144073	CUCGCUACGAGA AUGAGCUdTdT	1095	A-144074	AGCUCAUUCUCGUAGCGAGdTdT	1409	CUCGCUACGAGAAUGAGCU	1723

TABLE 7-continued

XDH Modified Sequences

Duplex Name	Sense Oligo Name	Sense OligoSeq	SEQ ID NO	Anti-sense Oligo Name	Antisense OligoSeq	SEQ ID NO	mRNA target sequence	SEQ ID NO
AD-71767	A-144075	CUGUCUCUCCGG CUGGUCAdTdT	1096	A-144076	UGACCAGCCGGAGAGACAGdTdT	1410	CUGUCUCUCCGGCUGGUCA	1724
AD-71768	A-144079	CCACGCCAAGAU CAAGUCAdTdT	1097	A-144080	UGACUUGAUCUUGGCGUGGdTdT	1411	CCACGCCAAGAUCAAGUCC	1725
AD-71769	A-144081	CAUAGAUACAUC AGAAGCUdTdT	1098	A-144082	AGCUUCUGAUGUAUCUAUGdTdT	1412	CAUAGAUACAUCAGAAGCU	1726
AD-71770	A-144085	UUUGUUUGUUUC AUUUCCAdTdT	1099	A-144086	UGGAAAUGAAACAAACAAAdTdT	1413	UUUGUUUGUUUCAUUUCCG	1727
AD-71771	A-144087	GCUGAUGAUGUU CCUGGGAdTdT	1100	A-144088	UCCCAGGAACAUCAUCAGCdTdT	1414	GCUGAUGAUGUUCCUGGGA	1728
AD-71772	A-144093	CAGUCUUUGCGA AGGAUAAdTdT	1101	A-144094	UUAUCCUUCGCAAAGACUGdTdT	1415	CAGUCUUUGCGAAGGAUAA	1729
AD-71773	A-144095	AAGGUUACUUGU GUUGGGAdTdT	1102	A-144096	UCCCAACACAAGUAACCUUdTdT	1416	AAGGUUACUUGUGUUGGGC	1730
AD-71774	A-144097	CAUAUCAUUGGU GCUGUGAdTdT	1103	A-144098	UCACAGCACCAAUGAUAUGdTdT	1417	CAUAUCAUUGGUGCUGUGG	1731
AD-71775	A-144099	UGGUUGCUGACA CCCCGGAdTdT	1104	A-144100	UCCGGGGUGUCAGCAACCAdTdT	1418	UGGUUGCUGACACCCCGGA	1732
AD-71776	A-144101	ACACACACAGAG AGCUGCAdTdT	1105	A-144102	UGCAGCUCUCUGUGUGUGUdTdT	1419	ACACACACAGAGAGCUGCC	1733
AD-71777	A-144103	GCCCAAGGGGUG AAAAUCAdTdT	1106	A-144104	UGAUUUUCACCCCUUGGGCdTdT	1420	GCCCAAGGGGUGAAAAUCA	1734
AD-71778	A-144105	UCACCUAUGAAG AACUACAdTdT	1107	A-144106	UGUAGUUCUUCAUAGGUGAdTdT	1421	UCACCUAUGAAGAACUACC	1735
AD-71779	A-144109	GAGGAUGCUAUA AAGAACAdTdT	1108	A-144110	UGUUCUUUAUAGCAUCCUCdTdT	1422	GAGGAUGCUAUAAAGAACA	1736
AD-71780	A-144111	CAACUCCUUUUA UGGACCUdTdT	1109	A-144112	AGGUCCAUAAAAGGAGUUGdTdT	1423	CAACUCCUUUUAUGGACCU	1737
AD-71781	A-144113	CCUGAGCUGAAG AUCGAGAdTdT	1110	A-144114	UCUCGAUCUUCAGCUCAGGdTdT	1424	CCUGAGCUGAAGAUCGAGA	1738
AD-71782	A-144115	AAAGGGGACCUA AAGAAGAdTdT	1111	A-144116	UCUUCUUUAGGUCCCCUUUdTdT	1425	AAAGGGGACCUAAAGAAGG	1739
AD-71783	A-144117	AGGGGUUUUCCG AAGCAGAdTdT	1112	A-144118	UCUGCUUCGGAAAACCCCUdTdT	1426	AGGGGUUUUCCGAAGCAGA	1740
AD-71784	A-144119	UAAUGUUGUGUC AGGGGAAdTdT	1113	A-144120	UUCCCCUGACACAACAUUAdTdT	1427	UAAUGUUGUGUCAGGGGAG	1741
AD-71785	A-144121	AGAUAUACAUCG GUGGCCAdTdT	1114	A-144122	UGGCCACCGAUGUAUAUCUdTdT	1428	AGAUAUACAUCGGUGGCCA	1742
AD-71786	A-144123	GCCAAGAGCACU UCUACCUdTdT	1115	A-144124	AGGUAGAAGUGCUCUUGGCdTdT	1429	GCCAAGAGCACUUCUACCU	1743
AD-71787	A-144125	GGAGACUCACUG CACCAUUdTdT	1116	A-144126	AAUGGUGCAGUGAGUCUCCdTdT	1430	GGAGACUCACUGCACCAUU	1744
AD-71788	A-144127	UUGCUGUUCCAA AAGGCGAdTdT	1117	A-144128	UCGCCUUUUGGAACAGCAAdTdT	1431	UUGCUGUUCCAAAAGGCGA	1745
AD-71789	A-144129	CGAGGCAGGGGA GAUGGAAdTdT	1118	A-144130	UUCCAUCUCCCCUGCCUCGdTdT	1432	CGAGGCAGGGGAGAUGGAG	1746
AD-71790	A-144131	AGCUCUUUGUGU CUACACAdTdT	1119	A-144132	UGUGUAGACACAAAGAGCUdTdT	1433	AGCUCUUUGUGUCUACACA	1747

TABLE 7-continued

XDH Modified Sequences

Duplex Name	Sense Oligo Name	Sense OligoSeq	SEQ ID NO	Anti-sense Oligo Name	Antisense OligoSeq	SEQ ID NO	mRNA target sequence	SEQ ID NO
AD-71791	A-144133	CAGAACACCAUG AAGACCAdTdT	1120	A-144134	UGGUCUUCAUGGUGUUCUGdTdT	1434	CAGAACACCAUGAAGACCC	1748
AD-71792	A-144135	CAGAGCUUUGUU GCAAAAAdTdT	1121	A-144136	UUUUUGCAACAAAGCUCUGdTdT	1435	CAGAGCUUUGUUGCAAAAA	1749
AD-71793	A-144137	AAAAUGUUGGGG GUUCCAAdTdT	1122	A-144138	UUGGAACCCCCAACAUUUUdTdT	1436	AAAAUGUUGGGGGUUCCAG	1750
AD-71794	A-144139	AGCAAACCGGAU UGUGGUUdTdT	1123	A-144140	AACCACAAUCCGGUUUGCUdTdT	1437	AGCAAACCGGAUUGUGGUU	1751
AD-71795	A-144141	UUCGAGUGAAGA GAAUGGAdTdT	1124	A-144142	UCCAUUCUCUUCACUCGAAdTdT	1438	UUCGAGUGAAGAGAAUGGG	1752
AD-71796	A-144143	AGGAGGCUUUGG AGGCAAAdTdT	1125	A-144144	UUUGCCUCCAAAGCCUCCUdTdT	1439	AGGAGGCUUUGGAGGCAAG	1753
AD-71797	A-144145	AAGGAGACCCGG AGCACUAdTdT	1126	A-144146	UAGUGCUCCGGGUCUCCUUdTdT	1440	AAGGAGACCCGGAGCACUG	1754
AD-71798	A-144147	UGUGGUGUCCAC GGCAGUAdTdT	1127	A-144148	UACUGCCGUGGACACCACAdTdT	1441	UGUGGUGUCCACGGCAGUG	1755
AD-71799	A-144149	UGGCCCUGGCUG CAUAUAAdTdT	1128	A-144150	UUAUAUGCAGCCAGGGCCAdTdT	1442	UGGCCCUGGCUGCAUAUAA	1756
AD-71800	A-144153	UGCGAUGCAUGC UGGACCAdTdT	1129	A-144154	UGGUCCAGCAUGCAUCGCAdTdT	1443	UGCGAUGCAUGCUGGACCG	1757
AD-71801	A-144155	CGUGAUGAGGAC AUGCUGAdTdT	1130	A-144156	UCAGCAUGUCCUCAUCACGdTdT	1444	CGUGAUGAGGACAUGCUGA	1758
AD-71802	A-144157	UAACUGGUGGCA GACAUCAdTdT	1131	A-144158	UGAUGUCUGCCACCAGUUAdTdT	1445	UAACUGGUGGCAGACAUCC	1759
AD-71803	A-144159	UCCCUUCCUGGC CAGAUAAdTdT	1132	A-144160	UUAUCUGGCCAGGAAGGGAdTdT	1446	UCCCUUCCUGGCCAGAUAC	1760
AD-71804	A-144161	UACAAGGUUGGC UUCAUGAdTdT	1133	A-144162	UCAUGAAGCCAACCUUGUAdTdT	1447	UACAAGGUUGGCUUCAUGA	1761
AD-71805	A-144163	AGACUGGGACAG UUGUGGAdTdT	1134	A-144164	UCCACAACUGUCCCAGUCUdTdT	1448	AGACUGGGACAGUUGUGGC	1762
AD-71806	A-144165	GCUCUUGAGGUG GACCACUdTdT	1135	A-144166	AGUGGUCCACCUCAAGAGCdTdT	1449	GCUCUUGAGGUGGACCACU	1763
AD-71807	A-144167	ACUUCAGCAAUG UGGGGAAdTdT	1136	A-144168	UUCCCCACAUUGCUGAAGUdTdT	1450	ACUUCAGCAAUGUGGGGAA	1764
AD-71808	A-144169	GAACACCCAGGA UCUCUCUdTdT	1137	A-144170	AGAGAGAUCCUGGGUGUUCdTdT	1451	GAACACCCAGGAUCUCUCU	1765
AD-71809	A-144171	CAGAGUAUUAUG GAACGAAdTdT	1138	A-144172	UUCGUUCCAUAAUACUCUGdTdT	1452	CAGAGUAUUAUGGAACGAG	1766
AD-71810	A-144173	AGCUUUAUUCCA CAUGGAAdTdT	1139	A-144174	UUCCAUGUGGAAUAAAGCUdTdT	1453	AGCUUUAUUCCACAUGGAC	1767
AD-71811	A-144175	ACAACUGCUAUA AAAUCCAdTdT	1140	A-144176	UGGAUUUUAUAGCAGUUGUdTdT	1454	ACAACUGCUAUAAAAUCCC	1768
AD-71812	A-144177	UCCCCAACAUCC GGGGCAAdTdT	1141	A-144178	UUGCCCCGGAUGUUGGGGAdTdT	1455	UCCCCAACAUCCGGGGCAC	1769
AD-71813	A-144179	UGGGCGGCUGUG CAAAACAdTdT	1142	A-144180	UGUUUUGCACAGCCGCCCAdTdT	1456	UGGGCGGCUGUGCAAAACC	1770
AD-71814	A-144181	AACCAACCUUCC CUCCAAAdTdT	1143	A-144182	UUUGGAGGGAAGGUUGGUUdTdT	1457	AACCAACCUUCCCUCCAAC	1771

TABLE 7-continued

XDH Modified Sequences

Duplex Name	Sense Oligo Name	Sense OligoSeq	SEQ ID NO	Anti-sense Oligo Name	Antisense OligoSeq	SEQ ID NO	mRNA target sequence	SEQ ID NO
AD-71815	A-144183	ACACGGCCUUCCGGGGCUUdTdT	1144	A-144184	AAGCCCCGGAAGGCCGUGUdTdT	1458	ACACGGCCUUCCGGGGCUU	1772
AD-71816	A-144185	UUGGGGGGCCCCAGGGGAUdTdT	1145	A-144186	AUCCCCUGGGGCCCCCCAAdTdT	1459	UUGGGGGGCCCCAGGGGAU	1773
AD-71817	A-144187	AUGCUCAUUGCCGAGUGCUdTdT	1146	A-144188	AGCACUCGGCAAUGAGCAUdTdT	1460	AUGCUCAUUGCCGAGUGCU	1774
AD-71818	A-144189	UGGAUGAGUGAAGUUGCAAdTdT	1147	A-144190	UUGCAACUUCACUCAUCCAdTdT	1461	UGGAUGAGUGAAGUUGCAG	1775
AD-71819	A-144191	AGUGACCUGUGGGAUGCCUdTdT	1148	A-144192	AGGCAUCCCACAGGUCACUdTdT	1462	AGUGACCUGUGGGAUGCCU	1776
AD-71820	A-144193	CCUGCAGAGGAGGUGCGGAdTdT	1149	A-144194	UCCGCACCUCCUCUGCAGGdTdT	1463	CCUGCAGAGGAGGUGCGGA	1777
AD-71821	A-144195	AGAAAAAACCUGUACAAAGdTdT	1150	A-144196	CUUUGUACAGGUUUUUUCUdTdT	1464	AGAAAAAACCUGUACAAAG	1778
AD-71822	A-144197	AAAGAAGGGGACCUGACAAdTdT	1151	A-144198	UUGUCAGGUCCCCUUCUUUdTdT	1465	AAAGAAGGGGACCUGACAC	1779
AD-71823	A-144199	ACACUUCAACCAGAAGCUUdTdT	1152	A-144200	AAGCUUCUGGUUGAAGUGUdTdT	1466	ACACUUCAACCAGAAGCUU	1780
AD-71824	A-144201	UUGAGGGUUUCACCUUGCAdTdT	1153	A-144202	UGCAAGGUGAAACCCUCAAdTdT	1467	UUGAGGGUUUCACCUUGCC	1781
AD-71825	A-144203	CAGAUGCUGGGAAGAAUGAdTdT	1154	A-144204	UCAUUCUUCCCAGCAUCUGdTdT	1468	CAGAUGCUGGGAAGAAUGC	1782
AD-71826	A-144205	UGCCUAGCAAGCUCUCAGUdTdT	1155	A-144206	ACUGAGAGCUUGCUAGGCAdTdT	1469	UGCCUAGCAAGCUCUCAGU	1783
AD-71827	A-144207	UAUCAUGCUCGGAAGAGUAdTdT	1156	A-144208	UACUCUUCCGAGCAUGAUAdTdT	1470	UAUCAUGCUCGGAAGAGUG	1784
AD-71828	A-144209	AGUGAGGUUGACAAGUUCAdTdT	1157	A-144210	UGAACUUGUCAACCUCACUdTdT	1471	AGUGAGGUUGACAAGUUCA	1785
AD-71829	A-144211	AACAAGGAGAAUUGUUGGAdTdT	1158	A-144212	UCCAACAAUUCUCCUUGUUdTdT	1472	AACAAGGAGAAUUGUUGGA	1786
AD-71830	A-144213	AAAAAGAGAGGAUUGUGCAdTdT	1159	A-144214	UGCACAAUCCUCUCUUUUUdTdT	1473	AAAAAGAGAGGAUUGUGCA	1787
AD-71831	A-144215	CAUAAUUCCCACCAAGUUUdTdT	1160	A-144216	AAACUUGGUGGGAAUUAUGdTdT	1474	CAUAAUUCCCACCAAGUUU	1788
AD-71832	A-144217	UUUGGAAUAAGCUUUACAAdTdT	1161	A-144218	UUGUAAAGCUUAUUCCAAAdTdT	1475	UUUGGAAUAAGCUUUACAG	1789
AD-71833	A-144219	AGUUCCUUUUCUGAAUCAAdTdT	1162	A-144220	UUGAUUCAGAAAAGGAACUdTdT	1476	AGUUCCUUUUCUGAAUCAG	1790
AD-71834	A-144221	AGGCAGGAGCCCUACUUCAdTdT	1163	A-144222	UGAAGUAGGGCUCCUGCCUdTdT	1477	AGGCAGGAGCCCUACUUCA	1791
AD-71835	A-144223	CAUGUGUACACAGAUGGCUdTdT	1164	A-144224	AGCCAUCUGUGUACACAUGdTdT	1478	CAUGUGUACACAGAUGGCU	1792
AD-71836	A-144225	UCUGUGCUGCUGACCCACAdTdT	1165	A-144226	UGUGGGUCAGCAGCACAGAdTdT	1479	UCUGUGCUGCUGACCCACG	1793
AD-71837	A-144229	GGCCAAGGCCUUCAUACCAdTdT	1166	A-144230	UGGUAUGAAGGCCUUGGCCdTdT	1480	GGCCAAGGCCUUCAUACCA	1794
AD-71838	A-144231	AAAAUGGUCCAGGUGGCCAdTdT	1167	A-144232	UGGCCACCUGGACCAUUUUdTdT	1481	AAAAUGGUCCAGGUGGCCA	1795

TABLE 7-continued

XDH Modified Sequences

Duplex Name	Sense Oligo Name	Sense OligoSeq	SEQ ID NO	Anti-sense Oligo Name	Antisense OligoSeq	SEQ ID NO	mRNA target sequence	SEQ ID NO
AD-71839	A-144233	GCCAGUAGAGCU CUGAAAAdTdT	1168	A-144234	UUUUCAGAGCUCUACUGGCdTdT	1482	GCCAGUAGAGCUCUGAAAA	1796
AD-71840	A-144235	AAUCCCCACCUC UAAGAUUdTdT	1169	A-144236	AAUCUUAGAGGUGGGGAUUdTdT	1483	AAUCCCCACCUCUAAGAUU	1797
AD-71841	A-144237	UAUAUCAGCGAG ACAAGCAdTdT	1170	A-144238	UGCUUGUCUCGCUGAUAUAdTdT	1484	UAUAUCAGCGAGACAAGCA	1798
AD-71842	A-144239	AGCACUAACACU GUGCCCAdTdT	1171	A-144240	UGGGCACAGUGUUAGUGCUdTdT	1485	AGCACUAACACUGUGCCCA	1799
AD-71843	A-144241	CAACACCUCUCC CACGGCUdTdT	1172	A-144242	AGCCGUGGGAGAGGUGUUGdTdT	1486	CAACACCUCUCCCACGGCU	1800
AD-71844	A-144243	UGCCUCUGUCAG CGCUGAAdTdT	1173	A-144244	UUCAGCGCUGACAGAGGCAdTdT	1487	UGCCUCUGUCAGCGCUGAC	1801
AD-71845	A-144245	ACCUCAAUGGAC AGGCCGUdTdT	1174	A-144246	ACGGCCUGUCCAUUGAGGUdTdT	1488	ACCUCAAUGGACAGGCCGU	1802
AD-71846	A-144247	GUCUAUGCGGCU UGUCAGAdTdT	1175	A-144248	UCUGACAAGCCGCAUAGACdTdT	1489	GUCUAUGCGGCUUGUCAGA	1803
AD-71847	A-144249	AGACCAUCUUGA AAAGGCUdTdT	1176	A-144250	AGCCUUUUCAAGAUGGUCUdTdT	1490	AGACCAUCUUGAAAAGGCU	1804
AD-71848	A-144251	UGGAACCCUACA AGAAGAAdTdT	1177	A-144252	UUCUUCUUGUAGGGUUCCAdTdT	1491	UGGAACCCUACAAGAAGAA	1805
AD-71849	A-144253	AAGAAUCCCAGU GGCUCCUdTdT	1178	A-144254	AGGAGCCACUGGGAUUCUUdTdT	1492	AAGAAUCCCAGUGGCUCCU	1806
AD-71850	A-144255	UGGGAAGACUGG GUCACAAdTdT	1179	A-144256	UUGUGACCCAGUCUUCCCAdTdT	1493	UGGGAAGACUGGGUCACAG	1807
AD-71851	A-144257	ACAGCUGCCUAC AUGGACAdTdT	1180	A-144258	UGUCCAUGUAGGCAGCUGUdTdT	1494	ACAGCUGCCUACAUGGACA	1808
AD-71852	A-144259	ACAGUGAGCUUG UCUGCCAdTdT	1181	A-144260	UGGCAGACAAGCUCACUGUdTdT	1495	ACAGUGAGCUUGUCUGCCA	1809
AD-71853	A-144261	ACUGGGUUUUAU AGAACAAdTdT	1182	A-144262	UUGUUCUAUAAAACCCAGUdTdT	1496	ACUGGGUUUUAUAGAACAC	1810
AD-71854	A-144263	ACCCAAUCUGGG CUACAGAdTdT	1183	A-144264	UCUGUAGCCCAGAUUGGGUdTdT	1497	ACCCAAUCUGGGCUACAGC	1811
AD-71855	A-144265	CAGCUUUGAGAC UAACUCAdTdT	1184	A-144266	UGAGUUAGUCUCAAAGCUGdTdT	1498	CAGCUUUGAGACUAACUCA	1812
AD-71856	A-144267	GGGAACCCCUUC CACUACUdTdT	1185	A-144268	AGUAGUGGAAGGGGUUCCCdTdT	1499	GGGAACCCCUUCCACUACU	1813
AD-71857	A-144269	CUUCAGCUAUGG GGUGGCUdTdT	1186	A-144270	AGCCACCCCAUAGCUGAAGdTdT	1500	CUUCAGCUAUGGGGUGGCU	1814
AD-71858	A-144271	CUUGCUCUGAAG UAGAAAUdTdT	1187	A-144272	AUUUCUACUUCAGAGCAAGdTdT	1501	CUUGCUCUGAAGUAGAAAU	1815
AD-71859	A-144273	AAAUCGACUGCC UAACAGAdTdT	1188	A-144274	UCUGUUAGGCAGUCGAUUUdTdT	1502	AAAUCGACUGCCUAACAGG	1816
AD-71860	A-144275	GAGAUCAUAAGA ACCUCCAdTdT	1189	A-144276	UGGAGGUUCUUAUGAUCUCdTdT	1503	GAGAUCAUAAGAACCUCCG	1817
AD-71861	A-144277	GCACAGAUAUUG UCAUGGAdTdT	1190	A-144278	UCCAUGACAAUAUCUGUGCdTdT	1504	GCACAGAUAUUGUCAUGGA	1818
AD-71862	A-144279	UGGAUGUUGGCU CCAGUCUdTdT	1191	A-144280	AGACUGGAGCCAACAUCCAdTdT	1505	UGGAUGUUGGCUCCAGUCU	1819

TABLE 7-continued

XDH Modified Sequences

Duplex Name	Sense Oligo Name	Sense OligoSeq	SEQ ID NO	Anti-sense Oligo Name	Antisense OligoSeq	SEQ ID NO	mRNA target sequence	SEQ ID NO
AD-71863	A-144281	UAAACCCUGCCA UUGAUAUdTdT	1192	A-144282	AUAUCAAUGGCAGGGUUUAdTdT	1506	UAAACCCUGCCAUUGAUAU	1820
AD-71864	A-144283	UUGGACAGGUGG AAGGGGAdTdT	1193	A-144284	UCCCCUUCCACCUGUCCAAdTdT	1507	UUGGACAGGUGGAAGGGGC	1821
AD-71865	A-144285	GGGCAUUUGUCC AGGGCCUdTdT	1194	A-144286	AGGCCCUGGACAAAUGCCCdTdT	1508	GGGCAUUUGUCCAGGGCCU	1822
AD-71866	A-144287	UUGGCCUCUUCA CCCUAGAdTdT	1195	A-144288	UCUAGGGUGAAGAGGCCAAdTdT	1509	UUGGCCUCUUCACCCUAGA	1823
AD-71867	A-144289	AGAGGAGCUACA CUAUUCAdTdT	1196	A-144290	UGAAUAGUGUAGCUCCUCUdTdT	1510	AGAGGAGCUACACUAUUCC	1824
AD-71868	A-144291	CCCCGAGGGGAG CCUGCAAdTdT	1197	A-144292	UUGCAGGCUCCCCUCGGGGdTdT	1511	CCCCGAGGGGAGCCUGCAC	1825
AD-71869	A-144293	CACACCCGUGGC CCUAGCAdTdT	1198	A-144294	UGCUAGGGCCACGGGUGUGdTdT	1512	CACACCCGUGGCCCUAGCA	1826
AD-71870	A-144295	ACCUACAAGAUC CCGGCAUdTdT	1199	A-144296	AUGCCGGGAUCUUGUAGGUdTdT	1513	ACCUACAAGAUCCCGGCAU	1827
AD-71871	A-144297	UUUGGCAGCAUC CCCAUUAdTdT	1200	A-144298	UAAUGGGGAUGCUGCCAAAdTdT	1514	UUUGGCAGCAUCCCCAUUG	1828
AD-71872	A-144301	CCUGCUCCGCGA CUGCCCAdTdT	1201	A-144302	UGGGCAGUCGCGGAGCAGGdTdT	1515	CCUGCUCCGCGACUGCCCC	1829
AD-71873	A-144303	CAACAAGAAGGC CAUCUAUdTdT	1202	A-144304	AUAGAUGGCCUUCUUGUUGdTdT	1516	CAACAAGAAGGCCAUCUAU	1830
AD-71874	A-144305	UAUGCAUCGAAG GCUGUUAdTdT	1203	A-144306	UAACAGCCUUCGAUGCAUAdTdT	1517	UAUGCAUCGAAGGCUGUUG	1831
AD-71875	A-144307	UGGAGAGCCGCC CCUCUUAdTdT	1204	A-144308	UAAGAGGGGCGGCUCUCCAdTdT	1518	UGGAGAGCCGCCCCUCUUC	1832
AD-71876	A-144309	UCCUGGCUGCUU CUAUCUUdTdT	1205	A-144310	AAGAUAGAAGCAGCCAGGAdTdT	1519	UCCUGGCUGCUUCUAUCUU	1833
AD-71877	A-144311	UUCUUUGCCAUC AAAGAUAdTdT	1206	A-144312	UAUCUUUGAUGGCAAAGAAdTdT	1520	UUCUUUGCCAUCAAAGAUG	1834
AD-71878	A-144313	CCAUCCGUGCAG CUCGAGAdTdT	1207	A-144314	UCUCGAGCUGCACGGAUGGdTdT	1521	CCAUCCGUGCAGCUCGAGC	1835
AD-71879	A-144315	AGCUCAGCACAC AGGUAAUdTdT	1208	A-144316	AUUACCUGUGUGCUGAGCUdTdT	1522	AGCUCAGCACACAGGUAAU	1836
AD-71880	A-144317	AAUAACGUGAAG GAACUCUdTdT	1209	A-144318	AGAGUUCCUUCACGUUAUUdTdT	1523	AAUAACGUGAAGGAACUCU	1837
AD-71881	A-144319	UUCCGGCUAGAC AGCCCUAdTdT	1210	A-144320	UAGGGCUGUCUAGCCGGAAdTdT	1524	UUCCGGCUAGACAGCCCUG	1838
AD-71882	A-144321	UGCCACCCCGGA GAAGAUAdTdT	1211	A-144322	UAUCUUCUCCGGGGUGGCAdTdT	1525	UGCCACCCCGGAGAAGAUC	1839
AD-71883	A-144323	AUCCGCAAUGCC UGCGUGAdTdT	1212	A-144324	UCACGCAGGCAUUGCGGAUdTdT	1526	AUCCGCAAUGCCUGCGUGG	1840
AD-71884	A-144325	GACAAGUUCACC ACCCUGUdTdT	1213	A-144326	ACAGGGUGGUGAACUUGUCdTdT	1527	GACAAGUUCACCACCCUGU	1841
AD-71885	A-144327	UGUGUGUCACUG GUGUCCAdTdT	1214	A-144328	UGGACACCAGUGACACACAdTdT	1528	UGUGUGUCACUGGUGUCCC	1842
AD-71886	A-144329	AGAAAACUGCAA ACCCUGAdTdT	1215	A-144330	UCAGGGUUUGCAGUUUUCUdTdT	1529	AGAAAACUGCAAACCCUGG	1843

TABLE 7-continued

XDH Modified Sequences

Duplex Name	Sense Oligo Name	Sense OligoSeq	SEQ ID NO	Anti-sense Oligo Name	Antisense OligoSeq	SEQ ID NO	mRNA target sequence	SEQ ID NO
AD-71887	A-144331	CUGGUCUGUGAG GGUCUAAdTdT	1216	A-144332	UUAGACCCUCACAGACCAGdTdT	1530	CUGGUCUGUGAGGGUCUAA	1844
AD-71888	A-144333	AAAGAGAGAGUC CUCAGCAdTdT	1217	A-144334	UGCUGAGGACUCUCUCUUUdTdT	1531	AAAGAGAGAGUCCUCAGCA	1845
AD-71889	A-144335	AGAGUCUUCUUG UGCUGCAdTdT	1218	A-144336	UGCAGCACAAGAAGACUCUdTdT	1532	AGAGUCUUCUUGUGCUGCC	1846
AD-71890	A-144337	GCCUUUGGGCUU CCAUGGAdTdT	1219	A-144338	UCCAUGGAAGCCCAAAGGCdTdT	1533	GCCUUUGGGCUUCCAUGGA	1847
AD-71891	A-144341	CAGAACAUGGAU CUAUUAAdTdT	1220	A-144342	UUAAUAGAUCCAUGUUCUGdTdT	1534	CAGAACAUGGAUCUAUUAA	1848
AD-71892	A-144343	UUAAAGUCACAG AAUGACAdTdT	1221	A-144344	UGUCAUUCUGUGACUUUAAdTdT	1535	UUAAAGUCACAGAAUGACA	1849
AD-71893	A-144345	AGACCUGUGAUU UGUCAAAdTdT	1222	A-144346	UUUGACAAAUCACAGGUCUdTdT	1536	AGACCUGUGAUUUGUCAAG	1850
AD-71894	A-144347	AGAUGGGAUUUG GAAGACAdTdT	1223	A-144348	UGUCUUCCAAAUCCCAUCUdTdT	1537	AGAUGGGAUUUGGAAGACA	1851
AD-71895	A-144349	AAGUGAAUGCAA UGGAAGAdTdT	1224	A-144350	UCUUCCAUUGCAUUCACUUdTdT	1538	AAGUGAAUGCAAUGGAAGA	1852
AD-71896	A-144351	AAGAUUUUGAUC AAAAAUAdTdT	1225	A-144352	UAUUUUUGAUCAAAAUCUUdTdT	1539	AAGAUUUUGAUCAAAAAUG	1853
AD-71897	A-144353	UGUAAUUUGUAA ACACAAUdTdT	1226	A-144354	AUUGUGUUUACAAAUUACAdTdT	1540	UGUAAUUUGUAAACACAAU	1854
AD-71898	A-144355	GAUAAGCAAAUU CAAAACUdTdT	1227	A-144356	AGUUUUGAAUUUGCUUAUCdTdT	1541	GAUAAGCAAAUUCAAAACU	1855
AD-71899	A-144357	ACUGUUAUGCCU AAAUGGUdTdT	1228	A-144358	ACCAUUUAGGCAUAACAGUdTdT	1542	ACUGUUAUGCCUAAAUGGU	1856
AD-71900	A-144359	UGAAUAUGCAAU UAGGAUAdTdT	1229	A-144360	UAUCCUAAUUGCAUAUUCAdTdT	1543	UGAAUAUGCAAUUAGGAUC	1857
AD-71901	A-144361	AUCAUUUUCUGU CUGUUUUdTdT	1230	A-144362	AAAACAGACAGAAAAUGAUdTdT	1544	AUCAUUUUCUGUCUGUUUU	1858
AD-71902	A-144363	UUAAUCAUGUAU CUGGAAUdTdT	1231	A-144364	AUUCCAGAUACAUGAUUAAdTdT	1545	UUAAUCAUGUAUCUGGAAU	1859
AD-71903	A-144365	AAUAGGGUCGGG AAGGGUUdTdT	1232	A-144366	AACCCUUCCCGACCCUAUUdTdT	1546	AAUAGGGUCGGGAAGGGUU	1860
AD-71904	A-144367	UUUGUGCUAUUC CCCACUUdTdT	1233	A-144368	AAGUGGGGAAUAGCACAAAdTdT	1547	UUUGUGCUAUUCCCCACUU	1861
AD-71905	A-144369	UUACUGGACAGC CUGUAUAdTdT	1234	A-144370	UAUACAGGCUGUCCAGUAAdTdT	1548	UUACUGGACAGCCUGUAUA	1862
AD-71906	A-144371	AACCUCAAGUUC UGAUGGUdTdT	1235	A-144372	ACCAUCAGAACUUGAGGUUdTdT	1549	AACCUCAAGUUCUGAUGGU	1863
AD-71907	A-144373	UGUCUGUCCUUU GAAGAGAdTdT	1236	A-144374	UCUCUUCAAAGGACAGACAdTdT	1550	UGUCUGUCCUUUGAAGAGG	1864
AD-71908	A-144375	AGGAUUCCCACA AACCUCUdTdT	1237	A-144376	AGAGGUUUGUGGGAAUCCUdTdT	1551	AGGAUUCCCACAAACCUCU	1865
AD-71909	A-144377	UAGAAGCUUAAA CCGAAGUdTdT	1238	A-144378	ACUUCGGUUUAAGCUUCUAdTdT	1552	UAGAAGCUUAAACCGAAGU	1866
AD-71910	A-144379	AAGUUACUUUAA AUCGUGUdTdT	1239	A-144380	ACACGAUUUAAAGUAACUUdTdT	1553	AAGUUACUUUAAAUCGUGU	1867

TABLE 7-continued

XDH Modified Sequences

Duplex Name	Sense Oligo Name	Sense OligoSeq	SEQ ID NO	Anti-sense Oligo Name	Antisense OligoSeq	SEQ ID NO	mRNA target sequence	SEQ ID NO
AD-71911	A-144381	UGCCUUCCUGUGAAAGCCUdTdT	1240	A-144382	AGGCUUUCACAGGAAGGCAdTdT	1554	UGCCUUCCUGUGAAAGCCU	1868
AD-71912	A-144383	CUGGCCUUCAAACCAAUGAdTdT	1241	A-144384	UCAUUGGUUUGAAGGCCAGdTdT	1555	CUGGCCUUCAAACCAAUGA	1869
AD-71913	A-144385	AACAGCAAAGCAUAACCUUdTdT	1242	A-144386	AAGGUUAUGCUUUGCUGUUdTdT	1556	AACAGCAAAGCAUAACCUU	1870
AD-71914	A-144387	UUGAAUCUAUACUCAAAUUdTdT	1243	A-144388	AAUUUGAGUAUAGAUUCAAdTdT	1557	UUGAAUCUAUACUCAAAUU	1871
AD-71915	A-144389	UUUUGCAAUGAGGCAGUGAdTdT	1244	A-144390	UCACUGCCUCAUUGCAAAAdTdT	1558	UUUUGCAAUGAGGCAGUGG	1872
AD-71916	A-144391	UGGGGUAAGGUUAAAUCCUdTdT	1245	A-144392	AGGAUUUAACCUUACCCCAdTdT	1559	UGGGGUAAGGUUAAAUCCU	1873
AD-71917	A-144393	UCUAACCAUCUUUGAAUCAdTdT	1246	A-144394	UGAUUCAAAGAUGGUUAGAdTdT	1560	UCUAACCAUCUUUGAAUCA	1874
AD-71918	A-144395	AUCAUUGGAAAGAAUAAAGdTdT	1247	A-144396	CUUUAUUCUUUCCAAUGAUdTdT	1561	AUCAUUGGAAAGAAUAAAG	1875
AD-71919	A-144397	AAUGAAACAAAUUCAAGGUdTdT	1248	A-144398	ACCUUGAAUUUGUUUCAUUdTdT	1562	AAUGAAACAAAUUCAAGGU	1876
AD-71920	A-144399	AGGUUAAUUGGAUCUGAUUdTdT	1249	A-144400	AAUCAGAUCCAAUUAACCUdTdT	1563	AGGUUAAUUGGAUCUGAUU	1877
AD-71921	A-144401	UUUGUGAAGCUGCAUAAAGdTdT	1250	A-144402	CUUUAUGCAGCUUCACAAAdTdT	1564	UUUGUGAAGCUGCAUAAAG	1878
AD-71922	A-144403	AGCAAGAUUACUCUAUAAUdTdT	1251	A-144404	AUUAUAGAGUAAUCUUGCUdTdT	1565	AGCAAGAUUACUCUAUAAU	1879
AD-71923	A-144405	UACAAAAAUCCAACCAACUdTdT	1252	A-144406	AGUUGGUUGGAUUUUUGUAdTdT	1566	UACAAAAAUCCAACCAACU	1880
AD-71924	A-144407	ACUCAAUUAUUGAGCACGUdTdT	1253	A-144408	ACGUGCUCAAUAAUUGAGUdTdT	1567	ACUCAAUUAUUGAGCACGU	1881
AD-71925	A-144409	UACAAUGUUCUAGAUUUCUdTdT	1254	A-144410	AGAAAUCUAGAACAUUGUAdTdT	1568	UACAAUGUUCUAGAUUUCU	1882
AD-71926	A-144411	UUCUUUCCCUUCCUCUUUAdTdT	1255	A-144412	UAAAGAGGAAGGGAAAGAAdTdT	1569	UUCUUUCCCUUCCUCUUUG	1883
AD-71927	A-144413	GAAGAGAAUAUUUGUAUUCdTdT	1256	A-144414	UAAUACAAAUAUUCUCUUCdTdT	1570	GAAGAGAAUAUUUGUAUUC	1884
AD-71928	A-144415	UUCCAAAUACUCUUUGAGUdTdT	1257	A-144416	ACUCAAAGAGUAUUUGGAAdTdT	1571	UUCCAAAUACUCUUUGAGU	1885
AD-71929	A-144417	UAUUUACAAAAAAGAUUAUdTdT	1258	A-144418	AUAAUCUUUUUUGUAAAUAdTdT	1572	UAUUUACAAAAAAGAUUAU	1886
AD-72020	A-144419	UAUGUUUAAUCUUUACAUUdTdT	1259	A-144420	AAUGUAAAGAUUAAACAUAdTdT	1573	UAUGUUUAAUCUUUACAUU	1887
AD-72021	A-144421	UUUGAAGCCAAAGUAAUUUdTdT	1260	A-144422	AAAUUACUUUGGCUUCAAAdTdT	1574	UUUGAAGCCAAAGUAAUUU	1888
AD-72022	A-144423	UUCCACCUAGAAAUGAUGAdTdT	1261	A-144424	UCAUCAUUUCUAGGUGGAAdTdT	1575	UUCCACCUAGAAAUGAUGC	1889
AD-72023	A-144425	UAUCAGUCCUGGCAUGGUAdTdT	1262	A-144426	UACCAUGCCAGGACUGAUAdTdT	1576	UAUCAGUCCUGGCAUGGUG	1890
AD-72024	A-144427	UGGCUCACCCCUAUAAUCAdTdT	1263	A-144428	UGAUUAUAGGGGUGAGCCAdTdT	1577	UGGCUCACCCCUAUAAUCC	1891

TABLE 7-continued

XDH Modified Sequences

Duplex Name	Sense Oligo Name	Sense OligoSeq	SEQ ID NO	Anti-sense Oligo Name	Antisense OligoSeq	SEQ ID NO	mRNA target sequence	SEQ ID NO
AD-72025	A-144429	AUCCCAGCACUU UGGGAGAdTdT	1264	A-144430	UCUCCCAAAGUGCUGGGAUdTdT	1578	AUCCCAGCACUUUGGGAGG	1892
AD-72026	A-144431	CUAAGGCAGGAG AAUUGCUdTdT	1265	A-144432	AGCAAUUCUCCUGCCUUAGdTdT	1579	CUAAGGCAGGAGAAUUGCU	1893
AD-72027	A-144433	UGCUUGAGCCCA GCAGUUUdTdT	1266	A-144434	AAACUGCUGGGCUCAAGCAdTdT	1580	UGCUUGAGCCCAGCAGUUU	1894
AD-72028	A-144435	UUGAGACCAGCC UGGGCAAdTdT	1267	A-144436	UUGCCCAGGCUGGUCUCAAdTdT	1581	UUGAGACCAGCCUGGGCAA	1895
AD-72029	A-144437	ACAUAGAGAGCU CCUGUCUdTdT	1268	A-144438	AGACAGGAGCUCUCUAUGUdTdT	1582	ACAUAGAGAGCUCCUGUCU	1896
AD-72030	A-144439	UCUUUAAAAAAA AUUUUUUdTdT	1269	A-144440	AAAAAAUUUUUUUUAAAGAdTdT	1583	UCUUUAAAAAAAAUUUUUU	1897
AD-72031	A-144441	UUAAUUAGUUGG UCUUGAUdTdT	1270	A-144442	AUCAAGACCAACUAAUUAAdTdT	1584	UUAAUUAGUUGGUCUUGAU	1898
AD-72032	A-144443	UAGUGCAUGCCU GUAGUCAdTdT	1271	A-144444	UGACUACAGGCAUGCACUAdTdT	1585	UAGUGCAUGCCUGUAGUCC	1899
AD-72033	A-144445	CCCAACUACUUG AAAGGCUdTdT	1272	A-144446	AGCCUUUCAAGUAGUUGGGdTdT	1586	CCCAACUACUUGAAAGGCU	1900
AD-72034	A-144447	CUGAGGUGGAGA GAUCAUUdTdT	1273	A-144448	AAUGAUCUCUCCACCUCAGdTdT	1587	CUGAGGUGGAGAGAUCAUU	1901
AD-72035	A-144449	UUUGAGCUCAGG AGGUUGAdTdT	1274	A-144450	UCAACCUCCUGAGCUCAAAdTdT	1588	UUUGAGCUCAGGAGGUUGA	1902
AD-72036	A-144451	UUGAGGCUGCAG UGAGCUAdTdT	1275	A-144452	UAGCUCACUGCAGCCUCAAdTdT	1589	UUGAGGCUGCAGUGAGCUA	1903
AD-72037	A-144455	CUCCUGCCUGAG CGACUGAdTdT	1276	A-144456	UCAGUCGCUCAGGCAGGAGdTdT	1590	CUCCUGCCUGAGCGACUGA	1904
AD-72038	A-144457	UGAGCAAGAUCU UGUCUCUdTdT	1277	A-144458	AGAGACAAGAUCUUGCUCAdTdT	1591	UGAGCAAGAUCUUGUCUCU	1905
AD-72039	A-144459	UGAAGAAAAAAA AAGAAAUdTdT	1278	A-144460	AUUUCUUUUUUUUUCUUCAdTdT	1592	UGAAGAAAAAAAAAGAAAU	1906
AD-72040	A-144461	UAAAAAUGCUGC UAUCAAAdTdT	1279	A-144462	UUUGAUAGCAGCAUUUUUAdTdT	1593	UAAAAAUGCUGCUAUCAAA	1907
AD-72041	A-144463	AAAAUCAAGCCC AACCAGAdTdT	1280	A-144464	UCUGGUUGGGCUUGAUUUUdTdT	1594	AAAAUCAAGCCCAACCAGA	1908
AD-72042	A-144465	AGAGGUAGAAGA GCCAAGAdTdT	1281	A-144466	UCUUGGCUCUUCUACCUCUdTdT	1595	AGAGGUAGAAGAGCCAAGA	1909
AD-72043	A-144467	AAGCCUGGGUUC UCAUCCUdTdT	1282	A-144468	AGGAUGAGAACCCAGGCUUdTdT	1596	AAGCCUGGGUUCUCAUCCU	1910
AD-72044	A-144469	CUAGCUCUGUCU CUUCUGUdTdT	1283	A-144470	ACAGAAGAGACAGAGCUAGdTdT	1597	CUAGCUCUGUCUCUUCUGU	1911
AD-72045	A-144473	UUGGACUGUCAA UUCCCCUdTdT	1284	A-144474	AGGGGAAUUGACAGUCCAAdTdT	1598	UUGGACUGUCAAUUCCCCU	1912
AD-72046	A-144475	CCUUCCUGUGAU CCAUUUUdTdT	1285	A-144476	AAAAUGGAUCACAGGAAGGdTdT	1599	CCUUCCUGUGAUCCAUUUU	1913
AD-72047	A-144481	UCUCACGUCUUC UGCUUUAdTdT	1286	A-144482	UAAAGCAGAAGACGUGAGAdTdT	1600	UCUCACGUCUUCUGCUUUA	1914
AD-72048	A-144485	UAUGACCUGAAA ACUCCAAdTdT	1287	A-144486	UUGGAGUUUUCAGGUCAUAdTdT	1601	UAUGACCUGAAAACUCCAG	1915

TABLE 7-continued

XDH Modified Sequences

Duplex Name	Sense Oligo Name	Sense OligoSeq	SEQ ID NO	Anti-sense Oligo Name	Antisense OligoSeq	SEQ ID NO	mRNA target sequence	SEQ ID NO
AD-72049	A-144487	UUACAUAAAGGAUCUGCAAdTdT	1288	A-144488	UUGCAGAUCCUUUAUGUAAdTdT	1602	UUACAUAAAGGAUCUGCAG	1916
AD-72050	A-144489	CAGCUAUCUAAGGCUUGGUdTdT	1289	A-144490	ACCAAGCCUUAGAUAGCUGdTdT	1603	CAGCUAUCUAAGGCUUGGU	1917
AD-72051	A-144493	AUGAUACCUGGGUCUAAUdTdT	1290	A-144494	UAUUAGACCCAGGUAUCAUdTdT	1604	AUGAUACCUGGGUCUAAUG	1918
AD-72052	A-144495	AACUCUGCUGAGAUCACCUdTdT	1291	A-144496	AGGUGAUCUCAGCAGAGUUdTdT	1605	AACUCUGCUGAGAUCACCU	1919
AD-72053	A-144497	ACCUCAAGUUUCUGCGGUUdTdT	1292	A-144498	AACCGCAGAAACUUGAGGUdTdT	1606	ACCUCAAGUUUCUGCGGUU	1920
AD-72054	A-144499	UUGGUAAAGAGAACAAGGAdTdT	1293	A-144500	UCCUUGUUCUCUUUACCAAdTdT	1607	UUGGUAAAGAGAACAAGGG	1921
AD-72055	A-144501	AAGAACAAACAUCCCUUUUdTdT	1294	A-144502	AAAAGGGAUGUUUGUUCUUdTdT	1608	AAGAACAAACAUCCCUUUU	1922
AD-72056	A-144503	UUUUAUUGCUCCAAAUGGUdTdT	1295	A-144504	ACCAUUUGGAGCAAUAAAAdTdT	1609	UUUUAUUGCUCCAAAUGGU	1923
AD-72057	A-144505	GAUUUAAUCCCUACAUGGUdTdT	1296	A-144506	ACCAUGUAGGGAUUAAAUCdTdT	1610	GAUUUAAUCCCUACAUGGU	1924
AD-72058	A-144507	GGUGCUGGGUGGACAAUGUdTdT	1297	A-144508	ACAUUGUCCACCCAGCACCdTdT	1611	GGUGCUGGGUGGACAAUGU	1925
AD-72059	A-144509	UGUCACUGUCACAUGCCUUdTdT	1298	A-144510	AAGGCAUGUGACAGUGACAdTdT	1612	UGUCACUGUCACAUGCCUU	1926
AD-72060	A-144511	UUCACUGUAUAAAUCCAAAdTdT	1299	A-144512	UUUGGAUUUAUACAGUGAAdTdT	1613	UUCACUGUAUAAAUCCAAC	1927
AD-72061	A-144513	ACCUUCUGCCAGAGAGAAUdTdT	1300	A-144514	AUUCUCUCUGGCAGAAGGUdTdT	1614	ACCUUCUGCCAGAGAGAAU	1928
AD-72062	A-144517	CAUGGAGGGAGGAUAGUGAdTdT	1301	A-144518	UCACUAUCCUCCCUCCAUGdTdT	1615	CAUGGAGGGAGGAUAGUGG	1929
AD-72063	A-144519	AAAUGAUAUAGUUGGACUAdTdT	1302	A-144520	UAGUCCAACUAUAUCAUUUdTdT	1616	AAAUGAUAUAGUUGGACUG	1930
AD-72064	A-144521	ACUGGUGCUUGAUGUCACUdTdT	1303	A-144522	AGUGACAUCAAGCACCAGUdTdT	1617	ACUGGUGCUUGAUGUCACU	1931
AD-72065	A-144523	CUAAUAAAUGAAACUGUCAdTdT	1304	A-144524	UGACAGUUUCAUUUAUUAGdTdT	1618	CUAAUAAAUGAAACUGUCA	1932

TABLE 8

XDH Single Dose (10 nM) Screen in Primary Human Hepatocytes

Duplex Name	AVG	STDEV	Position in NM_000379.3
AD-71930	82.8	4.5	18-36
AD-71931	56.8	3.9	36-54
AD-71932	60.4	4.8	69-87
AD-71933	49.5	5.4	86-104
AD-71934	65.8	15.1	104-122
AD-71935	50.8	6.2	119-137
AD-71936	65.3	19.6	154-172
AD-71937	94.4	19.3	172-190
AD-71938	90.8	1.8	188-206
AD-71939	80.7	9.1	205-223
AD-71940	67.2	2.3	223-241
AD-71941	70.8	23.2	239-257
AD-71942	49.6	8.9	273-291
AD-71943	50.3	3.7	291-309
AD-71944	52.9	6.5	308-326
AD-71945	79.4	5.0	325-343
AD-71946	92.7	0.1	342-360
AD-71947	50.1	17.5	358-376
AD-71948	65.1	19.8	376-394
AD-71949	87.7	0.1	392-410
AD-71950	44.1	2.8	410-428
AD-71951	70.6	5.2	425-443
AD-71952	43.5	4.0	444-462
AD-71953	85.3	1.7	459-477
AD-71954	56.1	7.2	477-495
AD-71955	80.7	22.7	495-513
AD-71956	81.6	9.6	510-528
AD-71957	69.1	8.1	529-547
AD-71958	60.6	2.7	544-562
AD-71959	79.8	12.1	563-581
AD-71960	68.4	16.0	580-598
AD-71961	51.1	2.0	596-614
AD-71962	52.0	0.7	630-648
AD-71963	56.9	3.6	647-665
AD-71964	52.4	3.4	663-681
AD-71965	90.2	29.6	680-698
AD-71966	53.2	5.5	697-715
AD-71967	54.5	4.2	714-732
AD-71968	53.3	3.2	731-749
AD-71969	57.0	4.7	748-766
AD-71970	72.1	10.5	765-783
AD-71971	60.0	4.2	783-801
AD-71972	86.7	6.8	799-817
AD-71973	89.1	7.0	816-834
AD-71974	65.9	6.2	834-852
AD-71975	62.3	16.3	852-870
AD-71976	75.7	22.3	869-887
AD-71977	69.5	17.5	884-902
AD-71978	112.9	17.2	902-920
AD-71979	88.8	12.2	919-937
AD-71980	47.2	20.4	936-954
AD-71981	51.9	9.6	953-971
AD-71982	46.5	1.2	969-987
AD-71983	58.2	14.4	986-1004
AD-71984	61.3	5.4	1005-1023
AD-71985	78.2	7.6	1021-1039
AD-71986	84.0	15.2	1037-1055
AD-71987	66.6	4.0	1056-1074
AD-71988	56.9	3.6	1071-1089
AD-71989	61.2	31.8	1088-1106
AD-71990	33.5	4.7	1105-1123
AD-71991	77.8	4.1	1123-1141
AD-71992	76.4	2.3	1141-1159
AD-71993	34.5	16.4	1158-1176
AD-71994	83.5	18.6	1173-1191
AD-71995	53.3	2.1	1190-1208
AD-71996	53.9	2.9	1207-1225
AD-71997	83.6	1.6	1225-1243
AD-71998	39.5	0.4	1242-1260
AD-71999	58.1	7.4	1259-1277
AD-72000	87.3	22.1	1277-1295
AD-72001	89.2	9.7	1292-1310
AD-72002	84.3	6.7	1310-1328
AD-72003	40.5	6.9	1326-1344
AD-72004	55.2	17.3	1343-1361

TABLE 8-continued

XDH Single Dose (10 nM) Screen in Primary Human Hepatocytes

Duplex Name	AVG	STDEV	Position in NM_000379.3
AD-72005	66.6	20.9	1362-1380
AD-72006	42.7	0.0	1378-1396
AD-72007	32.6	6.0	1394-1412
AD-72008	76.2	10.4	1411-1429
AD-72009	57.3	2.5	1428-1446
AD-72010	86.3	6.4	1445-1463
AD-72011	56.5	13.4	1463-1481
AD-72012	48.8	5.3	1481-1499
AD-72013	52.2	1.6	1496-1514
AD-72014	45.8	3.6	1515-1533
AD-72015	46.1	5.6	1530-1548
AD-72016	78.2	7.7	1548-1566
AD-72017	100.3	8.8	1564-1582
AD-72018	68.4	4.1	1583-1601
AD-72019	90.5	1.4	1598-1616
AD-71752	60.9	14.0	1617-1635
AD-71753	63.1	2.1	1634-1652
AD-71754	108.1	31.4	1667-1685
AD-71755	84.4	21.3	1685-1703
AD-71756	59.5	6.7	1702-1720
AD-71757	48.5	0.7	1718-1736
AD-71758	92.8	6.3	1734-1752
AD-71759	73.0	7.8	1752-1770
AD-71760	63.9	14.0	1770-1788
AD-71761	108.8	2.1	1787-1805
AD-71762	124.6	21.3	1803-1821
AD-71763	55.1	0.0	1819-1837
AD-71764	121.9	19.7	1836-1854
AD-71765	41.8	7.0	1854-1872
AD-71766	44.0	2.0	1872-1890
AD-71767	51.3	0.7	1889-1907
AD-71768	58.3	3.5	1921-1939
AD-71769	53.5	9.7	1939-1957
AD-71770	74.2	6.2	1973-1991
AD-71771	60.7	13.6	1991-2009
AD-71772	58.4	11.4	2040-2058
AD-71773	77.4	12.1	2057-2075
AD-71774	130.8	7.0	2075-2093
AD-71775	75.8	7.8	2091-2109
AD-71776	76.4	3.0	2110-2128
AD-71777	76.0	27.4	2126-2144
AD-71778	48.3	9.2	2142-2160
AD-71779	43.1	10.8	2177-2195
AD-71780	78.4	16.8	2194-2212
AD-71781	71.2	14.3	2210-2228
AD-71782	92.8	3.7	2228-2246
AD-71783	60.8	19.1	2244-2262
AD-71784	62.8	17.1	2263-2281
AD-71785	58.7	13.7	2280-2298
AD-71786	50.7	5.7	2295-2313
AD-71787	46.7	5.2	2314-2332
AD-71788	60.8	0.6	2331-2349
AD-71789	105.1	1.0	2347-2365
AD-71790	55.1	18.8	2364-2382
AD-71791	72.4	10.2	2381-2399
AD-71792	72.9	10.4	2399-2417
AD-71793	61.5	5.2	2414-2432
AD-71794	65.0	6.0	2431-2449
AD-71795	69.6	23.1	2448-2466
AD-71796	68.0	9.0	2467-2485
AD-71797	67.8	18.4	2483-2501
AD-71798	91.7	21.0	2500-2518
AD-71799	57.4	9.0	2517-2535
AD-71800	64.3	13.5	2550-2568
AD-71801	40.3	9.0	2567-2585
AD-71802	80.3	5.6	2586-2604
AD-71803	51.1	6.2	2602-2620
AD-71804	90.3	6.2	2618-2636
AD-71805	51.4	5.3	2637-2655
AD-71806	86.3	18.5	2654-2672
AD-71807	68.8	7.7	2670-2688
AD-71808	69.9	45.6	2686-2704
AD-71809	51.2	7.3	2705-2723
AD-71810	36.8	0.6	2722-2740
AD-71811	52.1	4.3	2739-2757

TABLE 8-continued

XDH Single Dose (10 nM) Screen in Primary Human Hepatocytes

Duplex Name	AVG	STDEV	Position in NM_000379.3
AD-71812	76.9	9.4	2754-2772
AD-71813	55.9	2.2	2773-2791
AD-71814	68.7	14.7	2788-2806
AD-71815	90.8	18.1	2805-2823
AD-71816	71.1	9.0	2823-2841
AD-71817	87.4	9.4	2840-2858
AD-71818	67.1	15.4	2858-2876
AD-71819	79.7	16.7	2875-2893
AD-71820	38.2	0.7	2891-2909
AD-71821	58.9	18.2	2909-2927
AD-71822	55.7	18.0	2924-2942
AD-71823	45.3	3.3	2941-2959
AD-71824	57.1	6.9	2958-2976
AD-71825	68.3	7.7	2977-2995
AD-71826	47.3	11.1	2993-3011
AD-71827	51.6	6.0	3011-3029
AD-71828	51.5	1.5	3026-3044
AD-71829	85.5	6.7	3044-3062
AD-71830	43.1	1.9	3062-3080
AD-71831	36.7	2.9	3079-3097
AD-71832	77.0	4.6	3095-3113
AD-71833	44.9	4.0	3112-3130
AD-71834	41.7	2.7	3129-3147
AD-71835	53.3	1.6	3146-3164
AD-71836	52.5	1.0	3164-3182
AD-71837	39.1	0.2	3197-3215
AD-71838	93.7	2.2	3215-3233
AD-71839	55.6	2.8	3230-3248
AD-71840	42.4	15.8	3247-3265
AD-71841	69.4	24.3	3266-3284
AD-71842	69.6	1.7	3281-3299
AD-71843	51.5	1.0	3298-3316
AD-71844	43.0	0.9	3316-3334
AD-71845	58.5	0.8	3333-3351
AD-71846	73.4	7.1	3350-3368
AD-71847	45.4	0.0	3366-3384
AD-71848	65.6	13.7	3384-3402
AD-71849	105.6	19.0	3401-3419
AD-71850	69.2	12.5	3419-3437
AD-71851	54.0	2.2	3434-3452
AD-71852	51.1	6.3	3452-3470
AD-71853	71.3	1.4	3470-3488
AD-71854	48.8	0.3	3487-3505
AD-71855	48.2	13.7	3502-3520
AD-71856	59.5	7.3	3521-3539
AD-71857	54.2	6.4	3538-3556
AD-71858	51.4	12.0	3555-3573
AD-71859	52.3	8.5	3570-3588
AD-71860	62.1	13.0	3588-3606
AD-71861	39.3	2.7	3606-3624
AD-71862	67.9	2.7	3621-3639
AD-71863	56.4	3.9	3639-3657
AD-71864	78.6	4.6	3657-3675
AD-71865	49.1	4.8	3672-3690
AD-71866	59.1	2.3	3690-3708
AD-71867	50.9	5.4	3706-3724
AD-71868	60.3	6.2	3724-3742
AD-71869	76.3	12.0	3740-3758
AD-71870	68.1	6.6	3758-3776
AD-71871	108.2	8.5	3776-3794
AD-71872	75.4	21.9	3808-3826
AD-71873	59.2	8.4	3826-3844
AD-71874	69.0	10.4	3842-3860
AD-71875	68.1	11.6	3859-3877
AD-71876	60.1	11.7	3876-3894
AD-71877	89.9	3.1	3893-3911
AD-71878	49.8	11.3	3912-3930
AD-71879	73.0	1.7	3928-3946
AD-71880	91.3	7.5	3944-3962
AD-71881	125.0	7.3	3962-3980
AD-71882	107.5	9.5	3979-3997
AD-71883	67.6	7.3	3995-4013
AD-71884	55.4	7.6	4013-4031
AD-71885	66.6	5.9	4029-4047
AD-71886	79.7	3.1	4048-4066

TABLE 8-continued

XDH Single Dose (10 nM) Screen in Primary Human Hepatocytes

Duplex Name	AVG	STDEV	Position in NM_000379.3
AD-71887	47.0	9.8	4063-4081
AD-71888	102.9	3.1	4080-4098
AD-71889	51.2	10.0	4098-4116
AD-71890	48.9	1.2	4114-4132
AD-71891	55.7	6.0	4150-4168
AD-71892	69.5	11.2	4165-4183
AD-71893	67.3	9.9	4183-4201
AD-71894	46.7	4.8	4200-4218
AD-71895	64.3	15.6	4218-4236
AD-71896	110.4	32.1	4233-4251
AD-71897	60.4	1.8	4250-4268
AD-71898	63.0	5.0	4269-4287
AD-71899	55.0	3.0	4285-4303
AD-71900	45.6	2.0	4303-4321
AD-71901	49.7	0.9	4319-4337
AD-71902	85.9	26.2	4336-4354
AD-71903	54.4	17.8	4352-4370
AD-71904	71.3	2.1	4369-4387
AD-71905	49.8	3.9	4386-4404
AD-71906	66.9	6.3	4404-4422
AD-71907	63.6	8.7	4422-4440
AD-71908	57.2	8.6	4438-4456
AD-71909	56.2	10.6	4456-4474
AD-71910	52.5	6.5	4471-4489
AD-71911	51.1	15.5	4489-4507
AD-71912	64.7	23.6	4506-4524
AD-71913	50.1	8.8	4524-4542
AD-71914	49.9	5.8	4541-4559
AD-71915	82.6	20.9	4558-4576
AD-71916	67.3	21.4	4574-4592
AD-71917	74.4	34.4	4592-4610
AD-71918	77.8	14.0	4607-4625
AD-71919	116.4	22.2	4626-4644
AD-71920	78.8	18.0	4641-4659
AD-71921	100.5	5.4	4659-4677
AD-71922	61.7	3.7	4676-4694
AD-71923	72.1	4.6	4694-4712
AD-71924	57.0	5.9	4710-4728
AD-71925	74.1	2.6	4728-4746
AD-71926	56.9	0.2	4743-4761
AD-71927	70.8	8.6	4761-4779
AD-71928	106.9	17.1	4777-4795
AD-71929	90.1	8.4	4795-4813
AD-72020	86.0	3.3	4811-4829
AD-72021	69.7	18.9	4828-4846
AD-72022	82.9	12.2	4845-4863
AD-72023	99.1	4.4	4864-4882
AD-72024	81.9	10.9	4881-4899
AD-72025	101.1	1.4	4896-4914
AD-72026	106.2	3.6	4915-4933
AD-72027	88.7	4.0	4930-4948
AD-72028	91.2	5.9	4947-4965
AD-72029	84.2	3.4	4965-4983
AD-72030	121.3	30.1	4981-4999
AD-72031	95.1	5.1	4999-5017
AD-72032	82.7	0.4	5017-5035
AD-72033	83.0	3.2	5034-5052
AD-72034	64.8	12.6	5051-5069
AD-72035	94.7	10.7	5068-5086
AD-72036	92.1	2.7	5083-5101
AD-72037	78.6	3.9	5118-5136
AD-72038	85.2	6.3	5134-5152
AD-72039	87.5	2.9	5152-5170
AD-72040	69.6	8.1	5170-5188
AD-72041	80.7	0.0	5186-5204
AD-72042	83.3	28.8	5202-5220
AD-72043	107.3	0.1	5220-5238
AD-72044	100.8	4.9	5237-5255
AD-72045	145.2	42.9	5272-5290
AD-72046	76.9	12.7	5288-5306
AD-72047	62.9	1.3	5339-5357
AD-72048	66.6	5.3	5372-5390
AD-72049	74.6	2.5	5391-5409
AD-72050	96.8	32.6	5407-5425
AD-72051	76.7	4.2	5440-5458

TABLE 8-continued

XDH Single Dose (10 nM) Screen in Primary Human Hepatocytes

Duplex Name	AVG	STDEV	Position in NM_000379.3
AD-72052	91.9	6.7	5459-5477
AD-72053	67.5	16.4	5474-5492
AD-72054	85.9	0.9	5491-5509
AD-72055	99.8	5.4	5510-5528
AD-72056	111.5	2.8	5525-5543
AD-72057	98.9	9.6	5544-5562
AD-72058	82.8	19.6	5560-5578
AD-72059	89.5	33.9	5578-5596
AD-72060	91.8	0.5	5595-5613
AD-72061	104.8	18.8	5612-5630
AD-72062	73.9	5.0	5644-5662
AD-72063	98.7	19.8	5663-5681
AD-72064	84.8	14.9	5678-5696
AD-72065	77.7	15.9	5695-5713

Example 4. In Vitro Determination IC_{50} Values of XDH iRNA Agents in Cynomolgus Monkey and Mouse Primary Hepatocytes

A series of siRNA-GalNAc3conjugates targeting an XDH gene were analyzed for target knockdown in cynomolgus monkey and mouse primary hepatocytes. The results are provided as IC_{50} (nM) in Table 9.

TABLE 9

XDH siRNA IC_{50} Values in Primary Cynomolgus Monkey and Mouse Hepatocytes

Duplex	Cyno	Mouse
AD-70042	0.0047	>10 nM
AD-70016	0.006	0.2345
AD-70030	0.0209	>10 nM
AD-70044	0.0224	>10 nM
AD-70050	0.0524	>10 nM
AD-70055	0.055	>10 nM
AD-70033	0.0569	0.1231
AD-70026	0.0614	0.1901
AD-70051	0.0859	>10 nM
AD-70052	0.1409	>10 nM
AD-70020	0.4017	4.4548
AD-70018	0.5446	0.8168
AD-70023	0.6569	0.4775
AD-70024	>10 nM	0.5886

Example 5. In Vivo Effect of Single Dose Administration of XDH iRNA Agents in Mice

A series of siRNA-GalNAc3conjugates targeting an XDH gene were designed and tested for the ability to knockdown expression of XDH mRNA in 6-8 week old C57BL/6 female mice (n=3 per group). A single 1 mg/kg dose of AD-70016, AD-70018, AD-70020, AD-70023, AD-70026, AD-70030, AD-70033; or PBS control, was administered subcutaneously to the mice on day 1. On day 10, the mice were sacrificed to assess knockdown of XDH mRNA in liver by RT-PCR. The effect of a single dose of the indicated agents are provided in Table 10. The data are presented as the XDH mRNA level remaining relative to PBS.

TABLE 10

XDH siRNA Single Dose (1 mg/kg) mRNA Knockdown in Mouse Liver

siRNA	Relative mRNA	St. Dev.
PBS	100.0	4.43
AD-70016	22.73	6.03
AD-70018	65.93	8.83
AD-70020	88.98	35.71
AD-70023	50.14	22.53
AD-70026	62.25	1.99
AD-70030	84.46	10.67
AD-70033	36.57	9.98

Example 6. In Vivo Effect of Increasing Single Dose Administration of XDH iRNA Agents in Mice

Male mice typically have higher serum levels of uric acid than female mice. Therefore, the ability of dsRNA agents targeting XDH to knockdown XDH was also assessed in male mice. Of the duplexes tested, AD-70016 was found to be most effective in decreasing expression of XDH mRNA and was subsequently analyzed in a dose-response study. C57BL/6 male mice, 6-8 weeks of age (n=3-4 per group) were administered a single dose of AD-70016 at 10, 3, 1, 0.3, or 0.1 mg/kg; or a PBS control. On day 10, the mice were sacrificed to assess knockdown of XDH mRNA in liver by RT-PCR. The effects of a single dose of AD-70016 at the concentrations indicated are provided in Table 10. The data are presented as the XDH mRNA level remaining relative to PBS.

TABLE 11

XDH siRNA AD-70016 Single Dose mRNA Knockdown Dose Response in Mouse Liver

mg/kg	Relative mRNA	St. Dev.
0 (PBS)	100.0	11.28
10	14.89	1.53
3	15.54	3.95
1	29.71	5.32
0.3	52.12	7.01
0.1	73.13	7.64

Further studies demonstrated the durability of knockdown by AD-70016 for at least 6 weeks post a single 10 mg/kg dose in C57BL/6 male mice.

Finally, in a single dose study using higher doses of GalNAc3 conjugated AD-70016 (3 mg/kg, 10 mg/kg, and 30 mg/kg), achieved robust knockdown of mRNA at day 10 (15.35%, 9.42%, and 8.29% remaining, respectively).

Example 7. In Vivo Effect of Increasing Single Dose Administration of XDH iRNA Agents in Rats

The AD-70016-GalNAc conjugate, which has a single nucleotide mismatch to the rat XDH sequence, was analyzed in a dose-response study in rats. Male Sprague-Dawley rats, 6-8 weeks of age (n=4 per group) were administered a single dose of AD-70016 at 15, 5, 1.5, 0.5, or 0.15 mg/kg; or a PBS control. On day 10, the rats were sacrificed to assess knockdown of XDH mRNA in liver by RT-PCR. The effects of a single dose of AD-70016 at the concentrations indicated are provided in Table 11. The data are presented as the XDH mRNA level remaining relative to PBS.

TABLE 12

XDH siRNA AD-70016 Single Dose mRNA Knockdown Dose Response in Rat Liver

mg/kg	Relative mRNA	St. Dev.
0 (PBS)	100	17.98
15	19.01	4.92
5	13.74	2.08
1.5	31.97	7.20
0.5	45.14	8.32
0.15	63.74	8.28

The results demonstrate that AD-70016 is effective in knocking down XDH expression in rats.

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments and methods described herein. Such equivalents are intended to be encompassed by the scope of the following claims.

SEQUENCE LISTING

The patent contains a lengthy "Sequence Listing" section. A copy of the "Sequence Listing" is available in electronic form from the USPTO web site (https://seqdata.uspto.gov/?pageRequest=docDetail&DocID=US11319539B2). An electronic copy of the "Sequence Listing" will also be available from the USPTO upon request and payment of the fee set forth in 37 CFR 1.19(b)(3).

We claim:

1. A double stranded ribonucleic acid (dsRNA) agent for inhibiting expression of a xanthine dehydrogenase (XDH) gene,

wherein said dsRNA agent comprises a sense strand and an antisense strand forming a double stranded region, wherein the sense strand comprises at least 15 contiguous nucleotides differing by no more than 3 nucleotides from the nucleotide sequence of nucleotides 271-309 of SEQ ID NO:1,

wherein the dsRNA agent comprises at least one nucleotide comprising a nucleotide modification, and

wherein the dsRNA agent further comprises a ligand.

2. The dsRNA agent of claim **1**, wherein substantially all of the nucleotides of said sense strand and substantially all of the nucleotides of said antisense strand comprise a nucleotide modification.

3. The dsRNA agent of claim **2**, wherein all of the nucleotides of said sense strand and all of the nucleotides of said antisense strand comprise a nucleotide modification.

4. The dsRNA agent of claim **3**, wherein the modified nucleotides are independently selected from the group consisting of a deoxy-nucleotide, a 3'-terminal deoxy-thymine (dT) nucleotide, a 2'-O-methyl modified nucleotide, a 2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an unlocked nucleotide, a conformationally restricted nucleotide, a constrained ethyl nucleotide, an abasic nucleotide, a 2'-amino-modified nucleotide, a 2'-O-allyl-modified nucleotide, 2'-C-alkyl-modified nucleotide, 2'-hydroxly-modified nucleotide, a 2'-methoxyethyl modified nucleotide, a 2'-O-alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, a non-natural base comprising nucleotide, a tetrahydropyran modified nucleotide, a 1,5-anhydrohexitol modified nucleotide, a cyclohexenyl modified nucleotide, a nucleotide comprising a phosphorothioate group, a nucleotide comprising a methylphosphonate group, a nucleotide comprising a 5'-phosphate, and a nucleotide comprising a 5'-phosphate mimic.

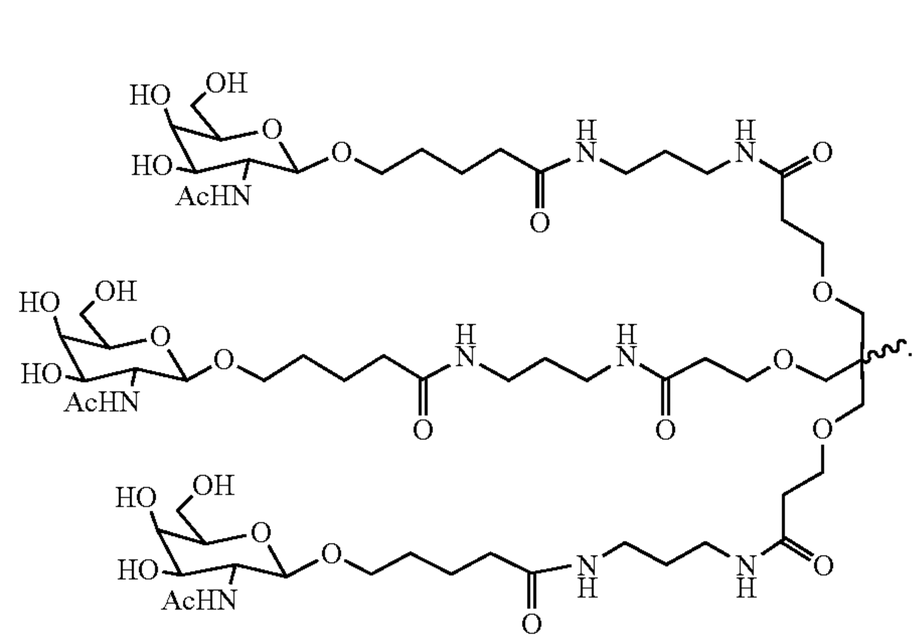
5. The dsRNA agent of claim **1**, wherein each strand is no more than 30 nucleotides in length.

6. The dsRNA agent of claim **1**, wherein at least one strand comprises a 3' overhang of at least 1 nucleotide; or at least 2 nucleotides.

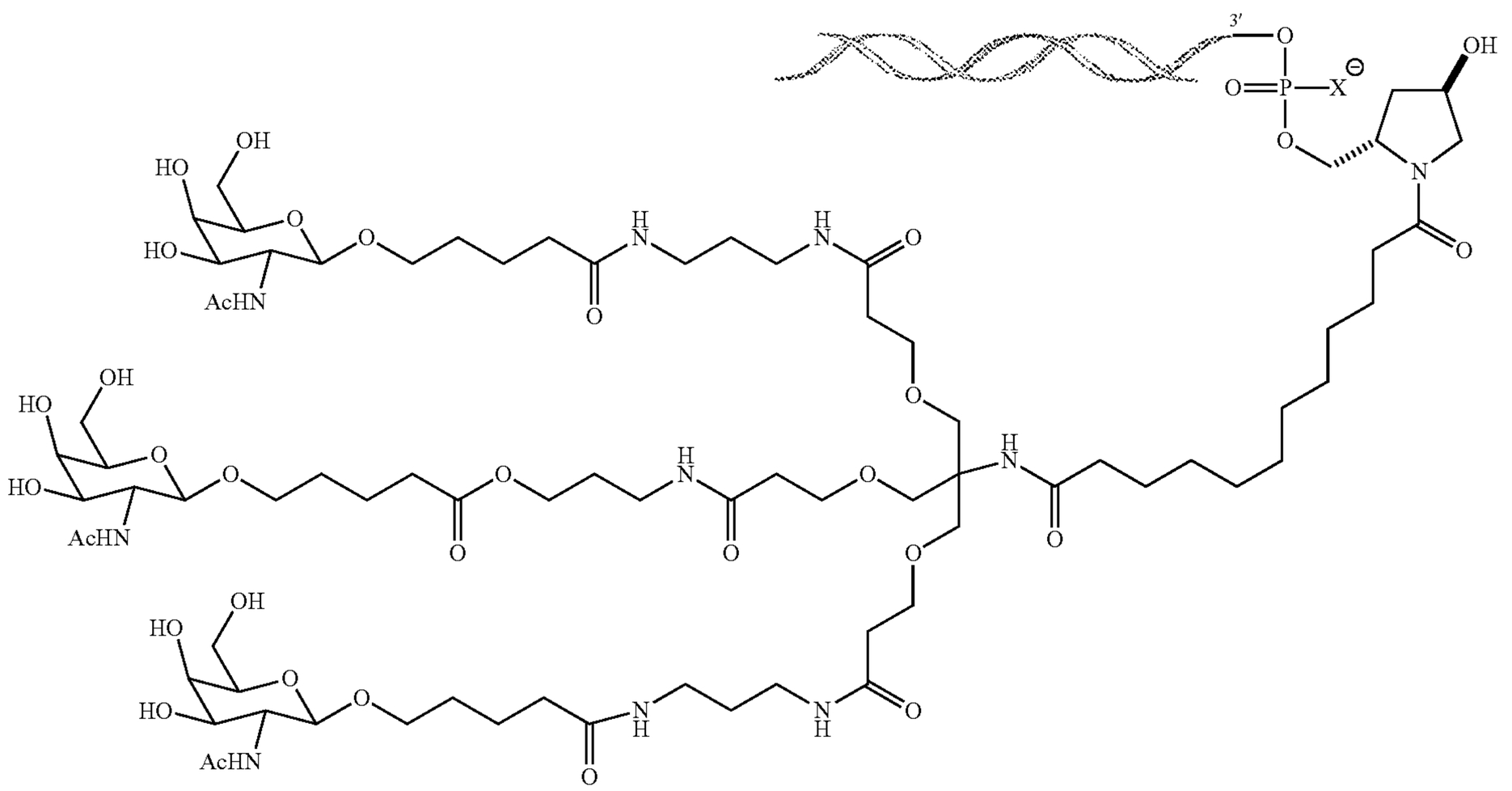
7. The dsRNA agent of claim **1**, wherein the ligand is conjugated to the 3' end of the sense strand of the dsRNA agent.

8. The dsRNA agent of claim **1**, wherein the ligand is an N-acetylgalactosamine (GalNAc) derivative.

9. The dsRNA agent of claim **8**, wherein the ligand is



10. The dsRNA agent of claim **9**, wherein the dsRNA agent is conjugated to the ligand as shown in the following schematic



and, wherein X is O or S.

11. The dsRNA agent of claim **10**, wherein X is O.

12. The dsRNA agent of claim **1**, wherein the double stranded region is 17-30 nucleotide pairs in length; 17-23 nucleotide pairs in length; 17-25 nucleotide pairs in length; 23-27 nucleotide pairs in length; 19-21 nucleotide pairs in length; or 21-23 nucleotide pairs in length.

13. The dsRNA agent of claim **1**, wherein each strand has 15-30 nucleotides; or 19-30 nucleotides.

14. The dsRNA agent of claim **1**, wherein said agent further comprises at least one phosphorothioate or methylphosphonate internucleotide linkage.

15. A pharmaceutical composition for inhibiting expression of an XDH gene comprising the dsRNA agent of claim **1**.

16. A method of inhibiting xanthine dehydrogenase (XDH) expression in a cell, the method comprising contacting the cell with the dsRNA agent of claim **1**, thereby inhibiting expression of the XDH gene in the cell.

17. A method of treating a subject having a disease or disorder that would benefit from reduction in XDH expression, the method comprising administering to the subject a therapeutically effective amount of the dsRNA agent of claim **1**, thereby treating said subject.

18. The dsRNA agent of claim **1**, wherein the antisense strand comprises at least 17 contiguous nucleotides differing by no more than three nucleotides from the nucleotide sequence of the complement of nucleotides 271-309 of SEQ ID NO:1.

19. The dsRNA agent of claim **18**, wherein the antisense strand comprises at least 17 contiguous nucleotides differing by no more than three nucleotides from any one of the nucleotide sequences selected from the group consisting of

(SEQ ID NO:689)
5′-UUGGCAGAAAAGUGGACGA-3′;
and

(SEQ ID NO:690)
5′-AUGGGGGCCAGGCAGGCAU-3′.

20. The dsRNA agent of claim **19**, wherein the sense strand and the antisense strand comprise nucleotide sequences selected from the group consisting of

(SEQ ID NO:375)
5′-UCGUCCACUUUUCUGCCAA-3′
and

(SEQ ID NO:689)
5′-UUGGCAGAAAAGUGGACGA-3′;
and

(SEQ ID NO:376)
5′-AUGCCUGCCUGGCCCCCAU-3′
and

(SEQ ID NO:690)
5′-AUGGGGGCCAGGCAGGCAU-3′.

* * * * *